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THE STATE OF WATER IN COLLOIDAL GELS: FREE AND BOUND WATER IN BREAD DOUGHS[1]

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In recent years considerable interest has been manifest regarding the state of water in both inorganic and organic colloidal systems. The systems investigated include such diverse materials as silica gels, lampblack and water, cold-resistant wheat and insects, living cells, dairy products, frozen foods, fresh and dried vegetables, cooked meats, and other colloidal gels. Of these, dough is one in which the state of water has been little studied. Some work has been done on flour-and-water suspensions in which the ratio in one study was 5 parts of flour to 50 parts of water and, in another, approximately 15 parts of flour to 85 parts of water. The water content in such cases was far in excess of that found in bread doughs, in which the ratio is about 55 parts of flour to 45 parts of water.

Much consideration has been given the tendency of certain doughs to become less plastic with continued mixing. The ability of doughs made from certain flours to withstand mixing and to show little loss of plasticity has been determined. This characteristic is frequently considered in testing flours for quality. The cause of this change in plasticity is not known but is frequently attributed to "the breaking down of the gluten"—a statement which apparently has little demonstrated scientific basis. The possibility of a change in the state of the water in a flour-water system with change in degree of mixing has been recognized. Some work has been done in an endeavor to determine whether or not such a change occurs, but no definite conclusions have been reached.

It was the purpose of this investigation to study further the state of water in bread dough, employing an adaptation of a technique found useful in the study of colloidal gels in a related field.

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In this study, a modification of the cryoscopic method was used. In studying freezing-point depressions, Schofield and Botelho Da Costa (1935) found that when the degree of supercooling was lessened they obtained freezing-point depressions only about one-half the value of those obtained by the Bouyoucos method using the Beckmann technique. Rather recently, Alexander and Shaw (1937 and 1937a) and Alexander, Shaw, and Mulkenhirn (1937), as a result of the findings obtained by Schofield and Botelho Da Costa (1935), started work on soil-water relationships. Alexander and Shaw considered the criticism of Kistler (1936) and of Briggs (1932) concerning the use of the Beckmann technique in the freezing-point-depression determinations and they concluded that failure to obtain equilibrium was a major criticism of this method. In determining the dielectric constant of soils they conceived the idea of employing that physical property in determining the freezing-point depressions of soil. They believed that the great difference in the dielectric constant of water and of ice should give a sharp change in the capacitance of a condenser containing water as one of the components, at the freezing or melting point.

Alexander and Shaw (1937 and 1937a) suggested that one may study the freezing-point depression by determining either the melting point or the freezing point, allowing sufficient time for equilibrium to be established. In most of their reported work, melting points were determined.

There are a number of theories regarding the structure of colloidal systems which may or may not explain the change in viscosity of flour-water systems.

An early view of colloidal solutions held by Hatschek (1927) was that it contained independent particles, each surrounded by a thick film of solvent, which in effect increased the particle size and also the viscosity. This view is not popular with some of the recent workers with wheat-flour suspensions and doughs, who find it difficult to explain the loss in plasticity on this basis.

On the other hand, the "brush-heap" picture presented by Newton and Cook (1930), by Skovholt and Bailey (1935), and by others offers a means of visualizing differences of flour strength and also changes in plasticity under various conditions. The micellar structure of the flour-water system is favored by a number of workers. This theory offers a possible explanation of differences in plasticity. It pictures the micelles in various degrees of aggregation, so that without appreciable change in degrees of hydration they may entrap more or less water to give a more or less viscous flour suspension.

Probably the first study of the "bound water" in flour-water systems was made by Newton and Cook (1930), using suspensions.

These workers measured bound water of wheat-flour suspensions in an attempt to obtain an index of flour quality. This provided a simple means for direct measurement of the approximate hydration of the colloidal constituents. In this study, the workers found no significant differences in degree of hydration between strong and weak flours. The results seem opposed to those obtained with plasticity studies but the authors suggested that the "brush-heap" structure made up of interwoven micelles offers a means of visualizing differences in gluten which would affect the strength.

Skovholt and Bailey (1935) were among the early workers to consider the bound water of a dough rather than of a fluid system. They devised a technique whereby the freezing point of the dough being studied could be determined. The method was rapid and it was possible to make a determination within a few minutes. Although the readings were somewhat erratic, the authors considered that when an average was taken of all of the readings, satisfactory results were obtained. The results indicated that the calculated bound-water values were not significantly different as a result of variable mixing and were approximately the same in doughs containing each of the three flours studied. The average bound-water value obtained was 51.4% of the total water present.

These workers did find significantly lowered freezing points of all doughs with an increase in the mixing time, due, apparently, to increasing concentration of solute. They were able to show an increase in the reducing sugars present with increased mixing which paralleled the decrease in freezing point. This increase was sufficient to account for from 13% to 16% of the observed decrease in freezing point when comparing doughs mixed for 1 and for 10 minutes. The authors considered that a hydrolysis of starch would probably be accompanied by an increase in the amount of intermediate products. These in turn might have some effect on the freezing point of the system. There may have been a similar hydrolysis of the protein but this would be difficult to prove.

Skovholt and Bailey believed that reactions of this type, in addition to increasing the soluble material of the system, also required water, thus further increasing the effect on the freezing point.

Experimental

In the present study, the purpose of which was to determine the state of water in doughs, it was proposed to determine the freezing or melting point of doughs without the supercooling characteristic of the Beckmann technique. The change in dielectric constant with the

melting or freezing of water as described by Alexander, Shaw, and Mulkenhirn (1937) was the basis for the work which follows.

The dielectric constant value was determined by measurement of the capacitance of a condenser under certain conditions. In this condenser or cell, the number of plates, the area of the plates, and the distance by which they were separated were fixed for each determination. The nature of the dielectric was the factor which varied, and accordingly the change noted in the capacity of the cell with change in temperature was due solely to the change in the physical state of the dielectric.

Materials and Method Used

The materials remained the same throughout the study. A commercial red winter wheat flour containing 10.1% of protein and 11.6% of moisture was used. The flour was stored in a sealed container at 5° ± 3°C. during the experiment. Sufficient flour for one mixing was removed to a small can with a tight-fitting lid and taken to the laboratory to reach room temperature before weighing. Distilled water was mixed with the flour to make the dough. One lot of c.p. sucrose was used throughout. The crystals were large and were added to the water in this form.

To study the effect of the length of the mixing period on the state of water in the dough, 100 g. of the flour were weighed, 60 g. of distilled water added, and these mixed in the Hobart-Swanson mixer for 1-, 3-, and 10-minute periods. The attachment of the mixer made 112 revolutions per minute.

When studying the depression of the freezing point resulting from the addition of sucrose, the sugar and distilled water were weighed, mixed, and the mixture weighed. This was agitated gently until all of the sucrose was dissolved. The solution was weighed again and, since any loss of weight was due to evaporation, the solution was made up to weight by the addition of distilled water. The flour was weighed and the two mixed immediately. All dough preparations containing sucrose were mixed for three minutes.

As in the doughs mixed for different lengths of time, 100 g. of flour and 60 g. of water were used. The flour was found to contain 11.6% moisture. Calculations to determine the amount of sucrose to give a 0.125, 0.25, and 0.5 molal solution were made on the basis of 71.6 g. of water. The amount of sucrose used for the solutions was 3.062 g., 6.125 g., and 12.25 g., respectively.

The circuit used for determining change in capacitance in the special condenser was essentially that of Alexander, Shaw, and Mulkenhirn (1937) and is shown in Figure 1. *O* is the oscillator, giving off radio

frequency waves at a frequency of 1797.5 kilocycles per second. L, C_s, and C_x form the receiving circuit, in which L is the inductance, C_s the calibrated condenser, and C_x the special condenser. The circuit was tuned to resonance with the oscillator.

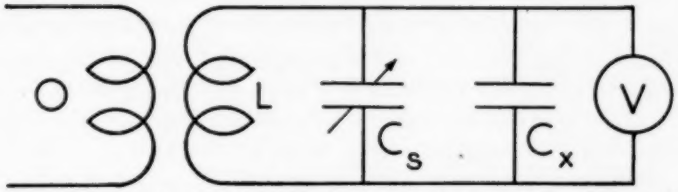

Fig. 1. Diagram of the circuit. O is the oscillator, L is the inductance of the receiving circuit, C_s is a calibrated condenser, and C_x is the special condenser in which the dough was placed.

The circuit was so designed that at a given capacity the receiver was "tuned" to the frequency of the transmitter. This is sometimes referred to as the resonance method of determining the dielectric constant. It is rather generally agreed that by this method small changes in conductance do not affect the capacitance readings. However, as pointed out by Alexander and Shaw (1937 and 1937a), an increase in the conductance of the dielectric, as occurred when the dough melted, resulted in a lowering of the sensitivity of the apparatus and unless the bias was set just right resulted in failure to measure small changes in capacitance of the special condenser.

The special condenser was made from a lusteroid 1.6 cm.-diameter test tube with the bottom cut off. This was inserted between two metal plates, 9.6 × 2 cm., bent to fit the tube and cemented to a larger glass tube so that their positions with relation to each other were fixed. To the metal plates, copper lead wires were soldered.

To prepare the condenser for a determination the lusteroid tube was drawn full of dough. A metal tube 1.3 cm. in outside diameter with a tapering point on one end was forced through the dough. Five such preparations were usually made from each dough and the melting point determined in from one to three of these. In each instance, a period of 10 minutes elapsed from the time the water was added to the flour to the filling of the tubes. That is, the dough mixed 1 minute stood for 9 minutes, that mixed 3 minutes stood for 7 minutes, and that mixed 10 minutes was placed in the tubes immediately. In general, it required about 10 minutes to fill the lusteroid tubes and insert the metal tubes into the dough. Thus about 20 minutes elapsed between the addition of the water to the flour and the placing of the dough in the kerosene at approximately −16°C.

As soon as the required number of tubes were ready, they were taken at once to the cold room, immersed in chilled kerosene, both at

approximately −16°C., and allowed to remain there until the melting point was to be determined. Then the tube to be used was taken from the kerosene, the kerosene emptied from the center metal tube, and the tube filled with water at approximately 35°C. This loosened the dough attached to the metal, the metal tube was readily withdrawn, and the dough remained frozen. The tube of dough was immediately inserted between the two metal plates, and the whole placed in a Dewar flask containing kerosene at about −16°C. The wires from the metal plates came out on opposite sides of the Dewar flask. A stopper containing three openings was placed in the Dewar flask, and the special condenser was held in place about a half inch below the level

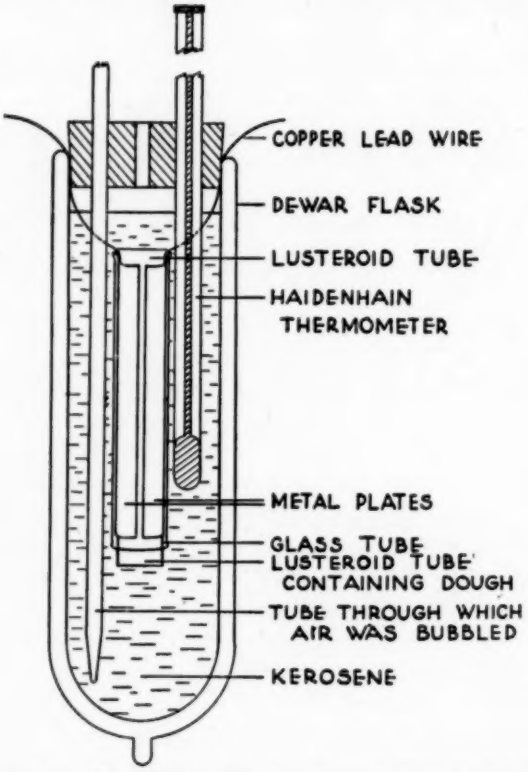

Fig. 2. Diagram of special cell, thermometer, glass tube, and stopper in place in Dewar flask.

of the kerosene. Through one opening in the stopper, a Haidenhain thermometer graduated in 0.01°C. with a range from −4.8° to +1.0°C. was inserted. A glass tube reaching almost to the bottom of the flask was inserted through the second opening. Cold dry air at a uniform pressure was passed through the glass tube to mix thoroughly the kerosene and thus maintain a uniform temperature throughout the flask.

The third opening was provided to permit free circulation of the air. No attempt was made to place the condenser in an exact position each time but the thermometer and condenser were adjusted so that they were close together near the center of the flask (Fig. 2).

The Dewar flask, with the condenser, thermometer, and glass tube in position, was then placed in an ice-salt bath. The special condenser containing the dough (C_x in Fig. 1) was connected in parallel with the calibrated variable condenser C_s of the receiver and the glass tube was connected with the source of the air under pressure. The receiver was tuned to the frequency of the oscillator and the temperature inside the flask was permitted to rise slowly. The change in capacitance of the special condenser (C_x) with change in temperature was noted. This was done by adjusting the calibrated variable condenser (C_s) to compensate for the change in the special condenser which in turn gave a method of measuring the change occurring in the special condenser.

The settings of the calibrated variable condenser were recorded approximately as follows for dough containing no sucrose: when first brought to the laboratory, then when the thermometer read, 7.5, 6.5, 5.0, 4.5, 4.0, 3.5, 3.0, 2.5, 2.0, 1.8, 1.7, 1.6, 1.4, 1.2, 1.1, 1.0, 0.9, 0.85, 0.8, 0.75, 0.7, 0.65, 0.6, 0.55, 0.5, 0.47, 0.44, 0.4, 0.37, 0.34, 0.3, 0.27, 0.24, 0.2, 0.17, 0.14, 0.1, 0.07, and 0.04 degrees below zero Centigrade, zero, and 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, and 1.0 degree above zero. When sucrose was added, the melting point was depressed so readings were taken at correspondingly lower points.

By plotting the values of the standard condenser against temperature a curve was obtained by means of which the melting point was determined.

Values of bound water were calculated by means of the widely used formula of Newton and Gortner (1922). This involves the use of freezing-point depressions with and without the addition of sucrose.

This formula assumes the formation of a hexahydrate of sucrose. Thus, for a 0.125 molal sucrose solution, 98.65% of the total water is not used in the formation of the sucrose hydrate; for a 0.25 molal sucrose solution, 97.3%; and for a 0.5 molal sucrose solution, 94.6%. The calculated values of the freezing point depressions (Δ) of the 0.125, 0.25, and 0.5 molal solutions are 0.236°, 0.478°, and 0.983°C., respectively. The formula for a 0.5 molal sucrose solution is:

$$\text{Percentage of bound water} = \frac{\Delta_a - (\Delta + K_m)}{\Delta_a - \Delta} \times 94.6$$

Δ = depression of freezing point of dough containing no sucrose;
Δ_a = depression of freezing point of dough containing sucrose;
K_m = molal constant for the depression of the freezing point.

This is given in a different form by Skovholt and Bailey (1935):

$$\text{Percentage of bound water} = \frac{\Delta_0 - \Delta_c}{\Delta_0} \times 94.6$$

Δ_0 = the observed difference in freezing point between doughs containing no added sugar and those containing added sugar sufficient to give a 0.5 molal concentration;

Δ_c = the calculated value for the freezing point depression due to 0.5 molal sucrose, assuming a hexahydrate formation.

The formulae of Newton and Gortner were recalculated by Grollman (1931), who proposed the following formula for use with a 0.5 molal sucrose solution:

$$\text{Percentage bound water} = \frac{\Delta_a - \left(\frac{1000}{946}\Delta + K_m\right)}{\Delta_a - \frac{1000}{946}\Delta} \times 94.6$$

Results

Throughout this study the data were recorded as change in capacitance with change in temperature as indicated by the settings of the calibrated condenser necessary to bring the system to resonance. These data were plotted as capacitance against temperature, with the "break" in the curve indicating the melting point. As a check on the method and the apparatus, ice made from distilled water was melted.

The curve in Figure 3 shows the change in capacitance with change in temperature of distilled water. The first straight-line portion reading from the left demonstrates a rise in temperature with no appreciable change in dielectric constant of the medium as indicated by change in capacitance. The vertical line obtained by plotting change in capacitance against change in temperature indicates that the melting point has been reached. This is according to the theory of the change of dielectric constant of water as it melts. Melting was not quite complete before the temperature of the water immediately surrounding the thermometer began to rise. This is shown by the slight slope of the line which follows the vertical portion. This was verified by removing the Dewar flask from the ice bath and noting the small shell of ice remaining.

The dough preparations made by mixing the flour and water for 1-, 3-, and 10-minute periods had almost identical melting points, the average melting point for each being -0.3°C. Plotting the data obtained by thawing the different doughs gave curves similar in shape.

The curves obtained by plotting change in capacitance against change in temperature for doughs mixed for one minute are shown in Figure 4.[2] Each curve represents the thawing of one tube of dough.

[2] Variations in the amount of dough in the tube and in the exact setting of the apparatus from time tot ime caused the total capacitance of the special condenser to vary. This accounts for the fact that the curves obtained by thawing a given series of doughs do not coincide.

Three different dough preparations are represented, duplicate samples having been used from one preparation. The horizontal line after the "break" indicates that thawing was complete. The length of time for melting and for the temperature to rise from $-1°$ to 0°C. are shown in

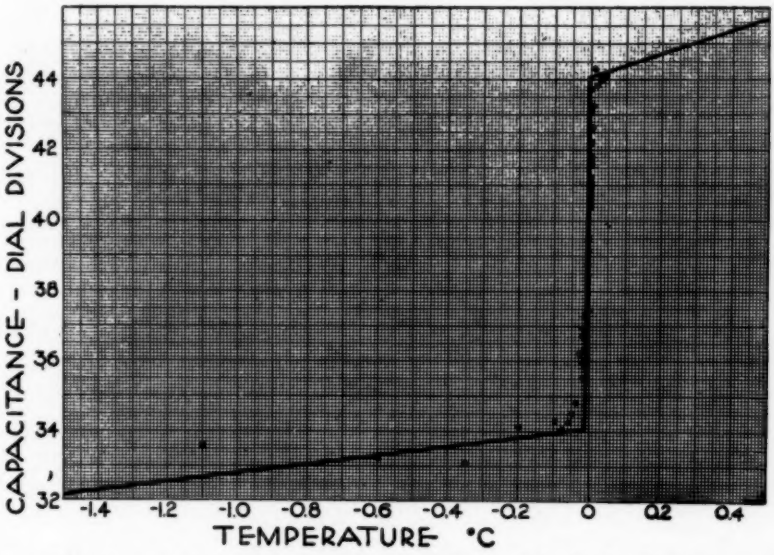

Fig. 3. Change in capacitance vs. temperature for distilled water during its elevation in temperature from the frozen to the fluid state.

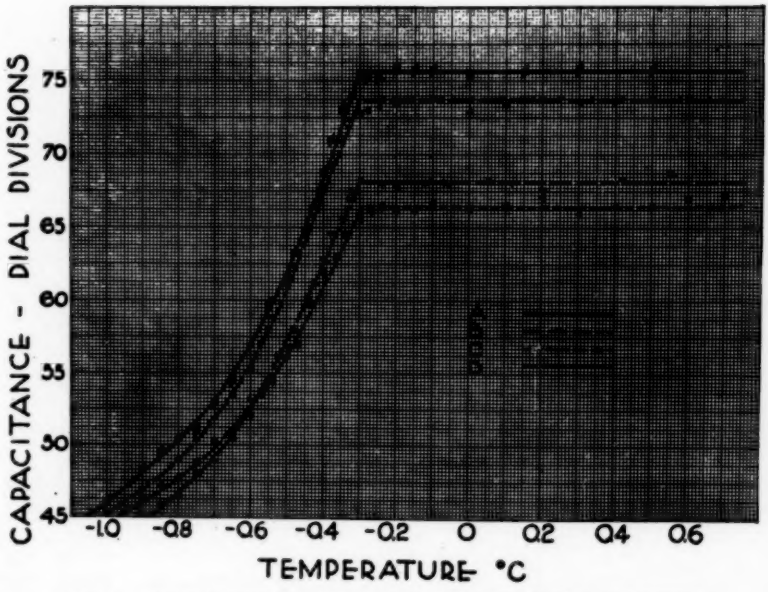

Fig. 4. Change in capacitance vs. temperature for doughs mixed one minute.

Table I. For complete thawing, a minimum of 4 hours was required. The significant range of temperature for these determinations was from −1° to 0°C. Approximately 2 hours and 30 minutes was the minimum time in which the temperature was satisfactorily brought through this range.

TABLE I

SUMMARY OF THAWING DATA FOR DOUGHS MIXED ONE MINUTE—WITHOUT SUCROSE

Curve	Dough preparation No.	Total time of thawing	Time to rise from −1° to 0°C.	Melting point—°C.
A	1	4 hrs. 6 min.	2 hrs. 27 min.	−0.30
B	2	4 hrs. 8 min.	2 hrs. 38 min.	−0.30
C	2	4 hrs. 30 min.	2 hrs. 39 min.	−0.30
D	3	4 hrs. 46 min.	2 hrs. 51 min.	−0.30
			Average	−0.300

The fact that there is no vertical portion in the curves obtained by melting the dough indicates that a solution and not water alone is being melted. Soluble materials in the flour have affected the freezing point of the water. As each increment of ice is frozen, the remaining solution is more concentrated and the freezing point is lowered. As the temperature is lowered more free water is frozen. When the process is reversed, the ice begins to melt. As the solution becomes more dilute, the melting point of the remaining ice is raised until finally melting is complete.

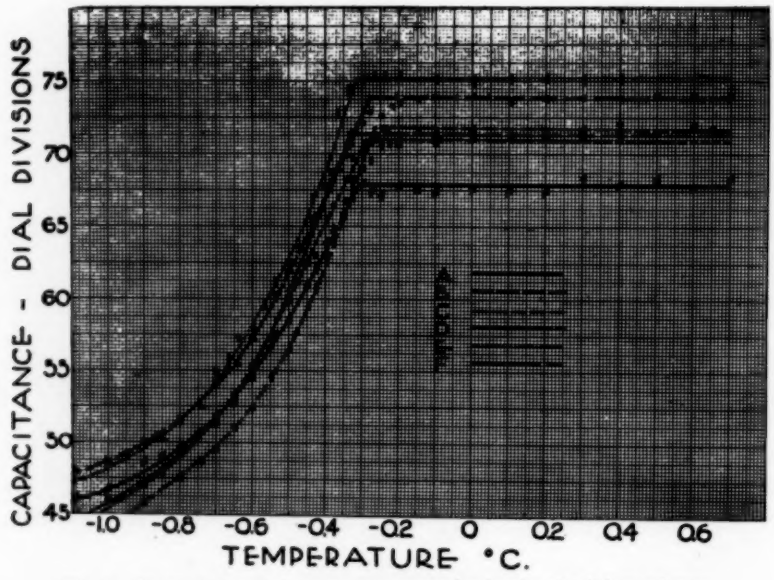

Fig. 5. Change in capacitance vs. temperature for doughs mixed three minutes.

Results of the plotting of data obtained by thawing doughs mixed for three minutes are recorded in Table II and in Figure 5. The satisfactory results obtained with shorter melting periods (curves *B* and *C*) indicate that possibly the dough mixed for three minutes thawed completely in less time than dough mixed for one minute. The results recorded are from three different dough preparations, using duplicate samples from each. The shape of the curves and the melting point do not differ significantly from those obtained with a one-minute mixing period.

TABLE II

SUMMARY OF THAWING DATA FOR DOUGHS MIXED THREE MINUTES—WITHOUT SUCROSE

Curve	Dough preparation No.	Total time of thawing	Time to rise from −1° to 0°C.	Melting point—°C.
A	1	3 hrs. 37 min.	1 hr. 21 min.	−0.31
B	1	3 hrs. 15 min.	1 hr. 59 min.	−0.28
C	2	3 hrs. 18 min.	1 hr. 13 min.	−0.27
D	2	3 hrs. 34 min.	1 hr. 23 min.	−0.29
E	3	4 hrs. 55 min.	3 hrs. 18 min.	−0.32
F	3	4 hrs. 22 min.	2 hrs. 15 min.	−0.33
			Average	−0.300

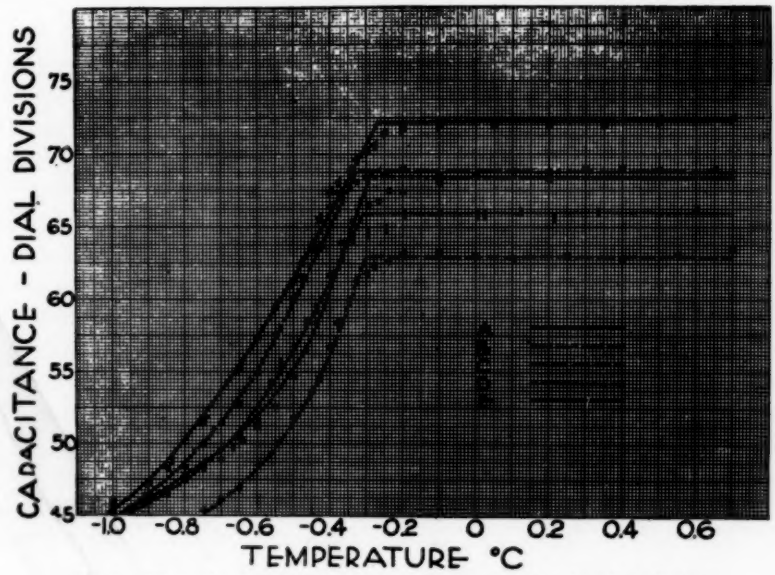

Fig. 6. Change in capacitance vs. temperature for doughs mixed ten minutes.

Figure 6 graphs the data obtained by thawing doughs mixed for a ten-minute period. These data are summarized in Table III. These represent two dough preparations using triplicate samples from one and

TABLE III

SUMMARY OF THAWING DATA FOR DOUGHS MIXED TEN MINUTES—WITHOUT SUCROSE

Curve	Dough preparation No.	Total time of thawing	Time to rise from −1° to 0°C.	Melting point—°C.
A	1	4 hrs. 10 min.	2 hrs. 41 min.	−0.27
B	1	4 hrs. 32 min.	2 hrs. 45 min.	−0.33
C	1	2 hrs. 26 min.	1 hr. 37 min.	−0.30
D	2	4 hrs. 10 min.	1 hr. 11 min.	−0.30
E	2	7 hrs. 28 min.	2 hrs. 44 min.	−0.31
			Average	−0.302

duplicate from the other. The shape of the curves indicates that the thawing was complete in each instance. The melting period for one determination (curve *C*) was extremely short and the time required for a rise from −1° to 0°C. was short in another of the determinations (curve *D*). The increase in mixing time gave a dough different in consistency from that obtained with less mixing. It may be that the smoother, more uniform dough resulting from the longer mixing time conducted the heat more efficiently, thus enabling it to reach a uniform temperature more quickly. Whether or not that is the explanation, it seemed that complete melting could be accomplished in less time with dough mixed for a longer period.

Addition of sucrose affected both the melting point and the shape of the curve. The results with a 0.125 molal sucrose solution are recorded graphically in Figure 7, representing duplicate samples from two different dough preparations. Not only was the melting point depressed but the rate of change in capacitance per unit change in temperature was less than that obtained when no sucrose was added. This tendency persisted on increasing the concentration of sucrose as is evident by comparing Figures 7, 8, and 9. With added sucrose, the time of thawing was decreased materially. The dough behaved more like the dough mixed for a longer period. The change in the shape of the curve was no doubt due to the smaller increment of water freezing at each temperature which resulted from the increased concentration of solutes.

The results with a 0.25 molal sucrose solution are shown in Figure 8. With increasing proportions of sucrose the dough thawed more rapidly and apparently temperature equilibrium was maintained with the more rapid rate of temperature increase.

Figure 9 shows the gentle slope of the curve obtained with 0.5 molal sucrose solution. The five sets of data involved two different dough preparations using triplicate samples from one and duplicate samples from the other.

Summaries of the results obtained with doughs mixed for three minutes are given in Table IV. These include the calculated "percent of bound water" computed from the melting-point data obtained by thawing the doughs mixed for three minutes, both with and without

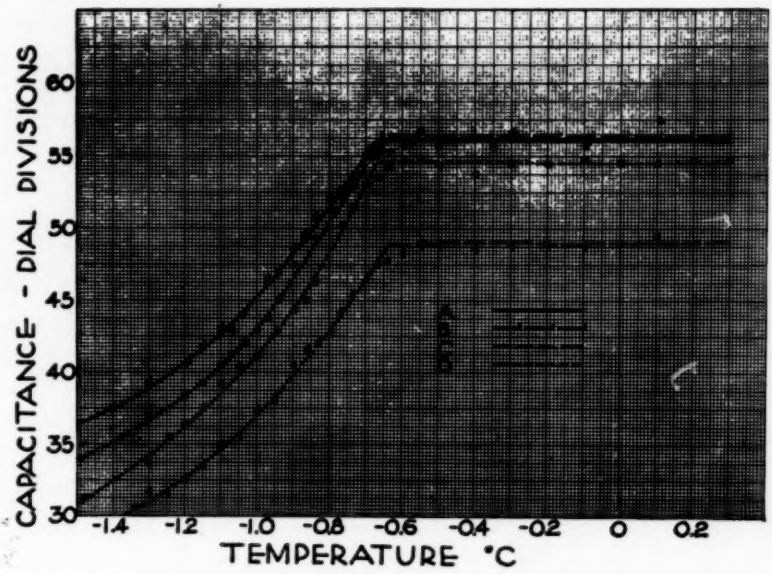

Fig. 7. Change in capacitance vs. temperature for doughs, 0.125 molal sucrose added.

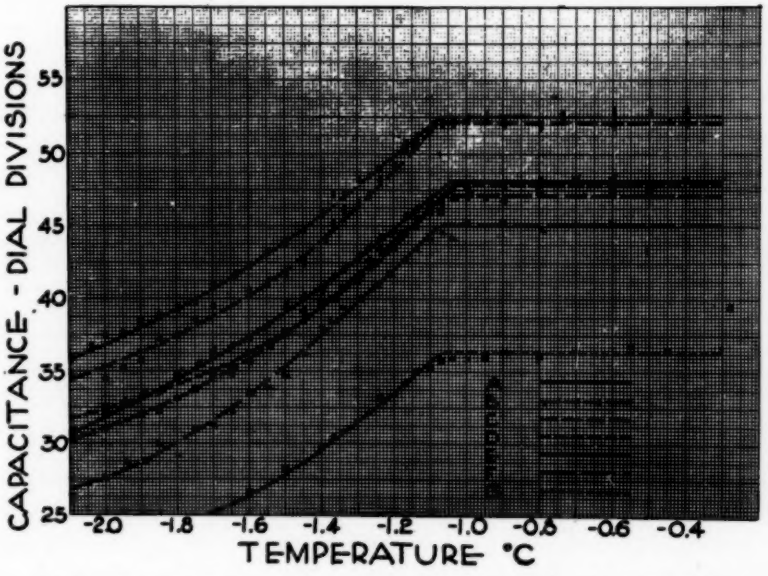

Fig. 8. Changes in capacitance vs. temperature for doughs, 0.25 molal sucrose added.

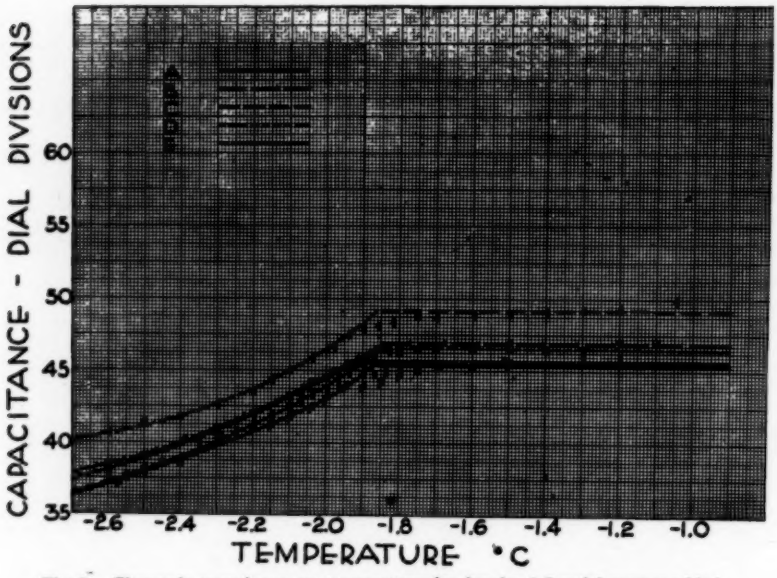

Fig. 9. Change in capacitance vs. temperature for doughs, 0.5 molal sucrose added.

the addition of sucrose. The formula of Newton and Gortner (1922), as given earlier, was used in calculating the percentage of bound water. The melting points obtained in this study were substituted for the freezing points indicated in the original formula, as the former were considered to be of the same value as the latter.

With a 0.125 molal sucrose solution:

$$\text{Percentage bound water} = \frac{0.665 - (0.3 + 0.236)}{0.665 - 0.3} \times 98.65 = 34.85$$

When a 0.25 molal sucrose solution was used:

$$\text{Percentage bound water} = \frac{1.064 - (0.3 + 0.478)}{1.064 - 0.3} \times 97.3 = 36.46$$

Using the results obtained with a 0.5 molal sucrose solution:

$$\text{Percentage bound water} = \frac{1.862 - (0.3 + 0.983)}{1.862 - 0.3} \times 94.6 = 35.06$$

Thus, using a 0.125, a 0.25, or a 0.5 molal sucrose solution, the results are in close agreement and there is no evidence that an increase of sucrose to give as much as a 0.5 molal solution tends to dehydrate or remove water from the flour particles.

TABLE IV

SUMMARY OF DATA FOR DOUGHS MIXED THREE MINUTES—WITH AND WITHOUT ADDED SUCROSE

Curve	Dough preparation No.	Total time of thawing	Time to rise from −1° to 0°C.	Melting point—°C.	
		NO SUCROSE ADDED			
A	1	3 hrs. 37 min.	1 hr. 21 min.	−0.31	
B	1	3 hrs. 15 min.	1 hr. 59 min.	−0.28	
C	2	3 hrs. 18 min.	1 hr. 13 min.	−0.27	
D	2	3 hrs. 34 min.	1 hr. 23 min.	−0.29	
E	3	4 hrs. 55 min.	3 hrs. 18 min.	−0.32	
F	3	4 hrs. 22 min.	2 hrs. 15 min.	−0.33	
			Average	−0.300	
Curve	Dough preparation No.	Total time of thawing	Time to rise from −1.5° to −0.5°C.	Melting point—°C.	Bound water, %
		0.125 MOLAL SUCROSE ADDED			
A	1	3 hrs. 19 min.	1 hr. 48 min.	−0.68	38.92
B	1	3 hrs. 41 min.	1 hr. 28 min.	−0.66	33.52
C	2	3 hrs. 29 min.	1 hr. 31 min.	−0.64	28.02
D	2	3 hrs. 10 min.	1 hr. 28 min.	−0.68	38.92
			Average	−0.665	34.85
Curve	Dough preparation No.	Total time of thawing	Time to rise from −1.5° to −0.5°C.	Melting point—°C.	Bound water, %
		0.25 MOLAL SUCROSE ADDED			
A	1	3 hrs. 33 min.	1 hr. 29 min.	−1.05	34.64
B	1	3 hrs. 21 min.	1 hr. 22 min.	−1.06	35.91
C	2	3 hrs. 4 min.	1 hr. 3 min.	−1.08	38.46
D	2	2 hrs. 42 min.	0 hr. 56 min.	−1.04	33.37
E	2	3 hrs. 27 min.	1 hr. 13 min.	−1.07	37.19
F	3	3 hrs. 19 min.	2 hrs. 0 min.	−1.08	38.46
G	3	3 hrs. 19 min.	1 hr. 32 min.	−1.07	37.19
			Average	−1.064	36.46
Curve	Dough preparation No.	Total time of thawing	Time to rise from −2.5° to −1.5°C.	Melting point—°C.	Bound water, %
		0.5 MOLAL SUCROSE ADDED			
A	1	2 hrs. 50 min.	44 minutes	−1.87	35.55
B	1	2 hrs. 35 min.	40 minutes	−1.85	34.34
C	1	2 hrs. 25 min.	36 minutes	−1.86	34.95
D	2	2 hrs. 44 min.	47 minutes	−1.85	34.34
E	2	2 hrs. 20 min.	63 minutes	−1.88	36.15
			Average	−1.862	35.06

Discussion

Inasmuch as supercooling occurs in the cryoscopic method as it is usually employed, this method has been open to criticism. Schofield and Botelho Da Costa (1935) showed that by reducing the extent of the supercooling in soils they obtained freezing-point depressions in some

instances, only one-half the magnitude of those obtained by applying the ordinary Beckmann technique. A second criticism of the cryoscopic method is failure to obtain temperature equilibrium. The system is constantly changing as the "freezing point" is being established, and time is an important factor. The temperature at the freezing point cannot be maintained for an appreciable length of time by the methods ordinarily employed. It is also impossible to determine the rate of freezing or the quantity of water freezing at the different stages as might sometimes be desirable.

The modification of the cryoscopic method as used in this study has several advantages over the original Beckmann technique, especially as applied to viscous systems. Supercooling no longer occurs since a melting point rather than a freezing point is established. The rate of rise in temperature of the system may be controlled so that a uniform temperature is maintained throughout and any given temperature may be held for the length of time desired. Furthermore, an approximation of the amount of water freezing or melting at the different temperatures may be determined by studying the resulting curves.

Study of the curves obtained by plotting capacitance against temperature should indicate the rate of melting, whether a pure solvent or a solution is being melted, something of the concentration of the solution, and the melting point. The extent to which the set-up used in this study met the above specifications was checked by using distilled water (Fig. 3).[3] The slight slope in the left-hand portion indicates that a small portion is melting, and the almost vertical portion indicates that it is a pure solvent rather than a solution that is melting. At the frequency used, the dielectric constant of ice does not change measurably with temperature, according to Murphy (1934). The point at which the great increase in capacitance occurs is, then, the melting point of the ice.

When the dough preparations were melted (Figs. 4, 5, and 6), the slope of the curve indicated that a solution was melting. The addition of sucrose (Figs. 7, 8, and 9) resulted in a greater slope which tended to flatten out with increased concentration of sugar.

The melting point of doughs as indicated by the curves is, if anything, slightly high. There was a tendency for the change in capacitance to lag behind the change in temperature whenever the rate of rise in temperature was not sufficiently slow. Accordingly, it appears that the minimum readings obtained are probably more nearly those of the true melting points than are the average readings.

When the formula of Grollman (1931) was used the results were

[3] It was impossible to use a shell of ice as thin as that of the dough because of the nature of ice; neither could it be thawed directly in the kerosene as was the dough. Hence, with this special condenser it was never possible to obtain complete melting of the ice before some rise in temperature occurred.

34.15%, 35.78%, and 34.41% bound water, respectively, for the 0.125, 0.25, and 0.5 molal sucrose solution. These differ only about 0.7% from the values obtained using the formula of Newton and Gortner (1922).

Thus using different concentrations of sucrose, no consistent, appreciable difference in bound water is shown whether one uses the formula of Newton and Gortner or that of Grollman.

That the process of freezing does not alter the dough so that the melting point differs significantly from the freezing point may be assumed from the work of Skovholt and Bailey (1935) which showed that "many replicated freezings and thawings of dough systems indicated no significant trend in successive values."

Robinson (1931) found in his work with insects that, once frozen, there was no apparent change in the state of water and determinations could be made as desired. This appeared to be true in this work on flour-water systems also, although none of the doughs were kept for more than a week.

With the exception of the work of Skovholt and Bailey (1935), previous determinations of bound water in flour-water systems had been made on systems containing a high percentage of water. In view of other work on bound water using gelatin, gum acacia, and other colloids, it seems highly probable that a system containing a high proportion of water would give very different percentages of bound water from one containing a low proportion. It would seem, then, that the results of the present study could best be compared with those of Skovholt and Bailey, whose work more closely approximates this research than any other. These workers used doughs made of flour and water mixed for varying lengths of time with and without the addition of sucrose. In every instance the freezing points of the doughs were much more depressed in the work of Skovholt and Bailey than in the present study. This great difference in the depression of the freezing point is undoubtedly due to the different methods used for the determinations. Since Schofield and Botelho Da Costa (1935) showed that decreasing the degree of the supercooling decreased the magnitude of the depression of the freezing point, eliminating supercooling entirely would undoubtedly have a similar effect. Skovholt and Bailey found it impossible to prevent supercooling with the method they used.

A significant difference in the freezing-point depressions of dough mixed for 1-, 2-, 3-, 4-, and 10-minute periods was also found by Skovholt and Bailey. This was attributed to the formation of soluble materials in the dough rather than to changes in the state of the water. They found no significant differences in percentage of bound water as calculated from the freezing point using 0.125, 0.25, and 0.5 molal

sucrose solutions. They did find an average of approximately 51.4% of the total water present as bound water, whereas in this study the average was 35.5%. Calculating this as hydration value, "that is, the amount of water held as bound per unit weight of solid material" which Skovholt and Bailey said may be a better method for comparison, one finds in this study an average total hydration of 28.6% as compared with 43.2% given by Skovholt and Bailey for the flour which had the protein value nearest that of the flour used here.

Comparisons with the results of Newton and Cook (1930) and of Kul'man and Golosova (1936) are difficult. Newton and Cook used suspensions of approximately 15% concentration and Kul'man and Golosova used 5 g. of absolutely dry flour in 50 g. of water. Hence, in both instances the percentage of water was exceedingly high as compared with that in doughs. If one accepts the prevailing idea that "bound water" is a relative term and that, as some workers have shown, the percentage of "bound water" is dependent upon the concentration of colloid in the system and upon the method used, one could hardly expect complete agreement in the results of the different workers with flour-water systems. Newton and Cook studied their suspensions by the cryoscopic method, and Kul'man and Golosova by Dumanskiĭ's refractometric method.

These workers agree with each other and with Skovholt and Bailey in finding no great difference in the water-binding capacity of weak and strong flours, but, as far as the percentage of bound water is concerned, they disagree with each other.

For winter wheat flour, durum wheat flour, and spring wheat flour, Kul'man and Golosova found from 44.4% to 54.4% of water bound or a total hydration of from 44.4% to 54.4%. Newton and Cook found only about 2% of the total water to be in the form of bound water or a hydration of the flour of about 11%. However, Newton and Cook did little work with untreated flour-water suspensions because of the relatively large errors involved.

Newton and Cook believed that bound water is a measure of true hydration. Kul'man and Golosova stated that there is a direct relationship between water-absorbing capacity of flour and its water-binding capacity.

The present study was not carried far enough to show the percentage of bound water at different levels of mixing. It does show no significant difference in the shape of the curves and in the freezing point for the doughs mixed for 1, 3, and 10 minutes. This does not agree with the work of Skovholt and Bailey, which showed a decrease in the freezing point with increase in mixing. This decrease was partially accounted for by the increase in soluble sugars. If there were

an appreciable increase in the amount of soluble materials in the dough preparations used in this study, this should be shown in the shape of the curve as well as in the melting point. The effect of the increased concentration of solute is clearly shown by the results obtained when sucrose was added.

Skovholt and Bailey made "all determinations that involved studies on the effect of mixing, immediately after the completion of mixing." They were able to remove the dough from the mixer, prepare it for and place it in the freezing bath in an elapsed time of one to two minutes, which means that determinations were started on doughs approximately 3, 4, 5, 6, and 12 minutes after the beginning of the mixing period. In the present study, approximately 20 minutes elapsed from the beginning of mixing to the placing of the dough in the cold kerosene. Steller, Markley, and Bailey (1935) had previously shown that over 70% of the total reducing sugar produced in a one-hour period is formed in the first 15 minutes. The doughs in the study here reported were at room temperature for approximately 20 minutes, sufficient time, according to Steller *et al.*, for the formation of considerable reducing sugar. Since each dough preparation was at room temperature for the same length of time, it seems probable that approximately the same percentage of reducing sugar may have been formed regardless of the mixing period.

The fact that there was no difference shown in this work in the freezing points of doughs mixed for different lengths of time, but of the same age when frozen, indicates no change had occurred in the state of water in the dough in consequence of variations in the degree of mixing. This is in agreement with the results of other workers who believe the "break-down" in bread doughs with overmixing must be accounted for by some means other than by changes in the hydration of flour particles. This "break-down" may result from changes in the "brush-heap" structure in which Newton and Cook (1930) as well as other workers found a possible explanation. As pointed out earlier, the idea of interwoven micelles offers a means of explaining the difference in the strengths of flours. Strong flours may be no more hydrated than weak flours, as the results of different workers indicate, but the difference in the aggregation of the micelles may result in different amounts of water being entrapped. Hence, strong flours may adsorb more water and thus have a higher initial viscosity than weaker flours. Changes in viscosity with age or with prolonged mixing may be the result of the squeezing out of the entrapped water due to shrinkage of the micelles or to decrease in aggregation of the micelles. This would mean no change in the amount of water "bound" and is in accord with the results obtained.

Summary

A method for determining the freezing or melting point of a system was devised which involved observing the changes in dielectric properties in progressing from the frozen to the melted state. The method is such that the system is in equilibrium and thus the stages in the freezing or thawing process may be observed. It is believed to be a suitable method for use in studying the state of water in highly viscous or plastic systems such as doughs.

Freezing points were determined for doughs mixed for 1-, 3-, and 10-minute periods. No significant differences were found. There was no indication of increase of solute with increase in mixing period under the conditions of dough treatment involved.

Average bound-water values for the dough mixed for three minutes were calculated. Calculations were based upon the depression of the freezing point of the doughs containing no sucrose and 0.125, 0.25, and 0.5 molal sucrose solutions. The calculated percentages of bound water at the three levels were 34.85, 36.46, and 35.06, respectively.

The average calculated bound water was 35.5% and the hydration capacity was 28.6% when calculated as bound water held per unit weight of dry matter.

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GAS PRODUCTION IN YEAST FERMENTATION AND ITS APPLICATIONS. I. VOLUMETRIC VS. MANOMETRIC METHODS FOR THE DETERMINATION OF GAS PRODUCTION

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A fermenting dough is characterized at least in part by the formation and liberation of carbon dioxide. It is apparent that both the total number of moles of gas produced and the time rate of their production are in some way a rather complex function of the yeast, the substrate, and the state of the system. This functional relationship is of supreme interest.

The study of gas production may be expected to yield information concerning not only the substrate (flour and ingredients other than yeast), and the yeast, but concerning the functional relationship itself. With this concept undoubtedly in mind, Bailey and Johnson (1924) and Bailey (1939) applied and improved a volumetric technique for the study of gas production under conditions of relatively constant pressure. Blish, Sandstedt, and Astleford (1932) and Sandstedt and Blish (1934) on the other hand adopted a manometric technique, that of measuring changes in pressure at relatively constant volume.

None of these workers, however, has published a comparison of the inherent errors, the complexity of applying necessary corrections, or the care required in maintenance of equipment in the respective methods. It is the purpose of this paper to attempt a detailed study of these factors.

Effects of Atmospheric Pressure

The manometric method.—A schematic diagram of the apparatus is given in Figure 1. A represents the vessel of volume V in which the

fermenting dough of non-gaseous volume v^* is placed. B indicates a simple mercury manometer of bore d. That segment of the left arm of the manometer between the mercury surface and the vessel A possesses an internal volume v', and h signifies the difference in height

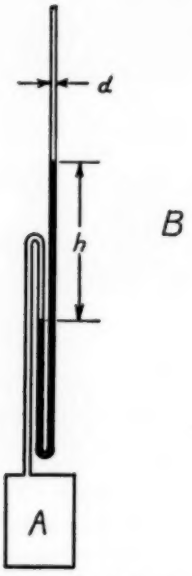

Fig. 1. Schematic diagram of manometric instrument.

between the mercury in both arms of the manometer—that is, the difference in pressure, say in millimeters of mercury, between the interior and the exterior of the vessel A. A is assumed immersed in a water bath at constant absolute temperature, T_{st}.

Throughout this paper we will adopt the following subscripts: st to represent standard conditions of pressure and temperature; s to represent the magnitude of the variable concerned at the time of sealing the equipment; r to represent the magnitude of the variable at the time a reading is taken. Let the atmospheric pressure be symbolized by P and let p represent the pressure of the gas produced by fermentation. For the sake of simplicity we will assume the gas law to hold. A more precise treatment might be attempted but the principles involved would remain unchanged.

At some time after sealing, consider n moles of carbon dioxide produced. Since the gas is assumed confined at constant free volume, $V' = V + v' - v^*$, n is proportional to the resulting pressure, p_r; that is

$$n = \frac{V'}{RT_{st}} p_r \tag{1}$$

where R is the gas constant in cubic centimeters—millimeters of mercury.
But:

$$p_r = h + P_r - P_s \tag{2}$$

where P_s is the partial pressure within the vessel due to atmospheric pressure at the time of sealing and p_r is the partial pressure of the carbon dioxide formed by fermentation during the interval sealing to reading. Obviously if there is no change in P during the course of measurement, $\Delta P = P_r - P_s = 0$, and n is simply proportional to h. If this condition is not fulfilled then an appropriate correction must be made to h if the proportionality of n to h is to be preserved. This correction is additive and is equal to ΔP; that is, the percentage correction in h associated with a given percentage change in P is equal to $100\Delta P/h$. It is to be noted that P_{st} does not occur in any of these relationships, and that therefore the manometric method is independent of any arbitrarily selected standard state of pressure so long as one does not calculate the volume corresponding to any given h. We may combine equations 1 and 2 to

$$n = \frac{V'}{RT_{st}}(h + P_r - P_s) \tag{3}$$

But

$$n = \frac{v_{st}}{RT_{st}} P_{st} \tag{4}$$

where v_{st} represents the volume of carbon dioxide under conditions of standard temperature and pressure, corresponding to the n moles liberated. Combining equations 3 and 4 we finally obtain:

$$v_{st} = \frac{V'}{P_{st}}(h + P_r - P_s) \tag{5}$$

where h and P are expressed in millimeters and v_{st} and V' in cubic centimeters.

The volumetric method.—Figure 2 presents a schematic diagram of the apparatus. A represents a vessel of any convenient volume V in which the fermenting dough of non-gaseous volume v^* is held. B is a rubber tube of internal volume v' connecting the vessel A to a Hempel gas burette C. D is some liquid preferably of low density, of low vapor pressure, and which does not absorb carbon dioxide. E is a leveling bulb so held that the liquid both in the burette and in the leveling bulb is at an identical level whenever a reading of the volume of gas produced is made. F is a rubber tube connecting burette to leveling bulb. A is assumed immersed in a constant temperature water bath at T_{st}.

The fixed total volume of air confined in the system at the time of sealing is then

$$V' = V + v' - v^* \tag{6}$$

It is this residual air in the system that complicates the corrections in the volumetric method. For if the atmospheric pressure changes from

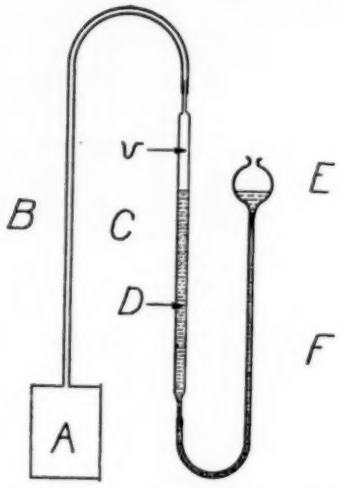

Fig. 2. Schematic diagram of volumetric instrument.

time of sealing to time of reading then the residual air itself will suffer a change of volume thereby causing an error in the volume of carbon dioxide v_r read from the burette. That is

$$V' + v_s = \frac{P_r}{P_s}(V' + v_r) \tag{7}$$

where v_s is the volume corrected for this error

$$v_s = \frac{P_r - P_s}{P_s} V' + \frac{P_r}{P_s} v_r \tag{8}$$

But

$$v_{st} = \frac{P_s}{P_{st}} v_s \tag{9}$$

where v_{st} is the reading corrected to standard pressure. Therefore:

$$v_{st} = \frac{P_r - P_s}{P_{st}} V' + \frac{P_r}{P_{st}} v_r \tag{10}$$

It is interesting to compare this relationship to equation 5, the final

expression for correcting the manometric reading for changes in atmospheric pressure. We rearrange equation 5 slightly for this purpose:

$$v_{st} = \frac{P_r - P_s}{P_{st}} V' + \frac{V'}{P_{st}} h \qquad (5')$$

We see here that the manometric method possesses a tangible advantage over the volumetric. For when $P_r = P_s \neq P_{st}$ the manometric method requires no corrections while the volumetric method requires one; and for all other cases where $P_r \neq P_s \neq P_{st}$ corrections for the manometric method still require one less operation.

Effects of Room Temperature

The manometric method.—Conventionally pressure is expressed in terms of millimeters of mercury and hence we are able to write $p_r = h$. But since in actual units of pressure p_r is expressed in dynes per square centimeters:

$$p_r = h\delta g \qquad (11)$$

where δ is the density of mercury at the temperature under consideration and g is the acceleration due to gravity. For a given value of p_r:

$$\frac{100\Delta h}{h} = -\frac{100\Delta\delta}{\delta} \qquad (12)$$

and the percentage variation in h is equal to the percentage variation in δ due to changes in room temperature. But a change of even 100°C. produces less than a 2% change in δ. Therefore we may consider the manometric method free from errors of this sort.

The volumetric method.—Variations in room temperature during the course of the determination may be considered the most significant source of error in the volumetric procedure. One must view the total volume of carbon dioxide and air confined in the connecting tube B and in the gas burette D. Since the internal volume of the connecting tube B is v', the volume of gas and air affected by variations in absolute room temperature, T, is equal to $v_r + v'$. We may write

$$v' + v_s = \frac{T_s}{T_r}(v' + v_r) \qquad (13)$$

where v_s is the reading corrected to the temperature at sealing

$$v_s = \frac{T_s - T_r}{T_r} v' + \frac{T_s}{T_r} v_r \qquad (14)$$

But

$$v_{st} = \frac{T_{st}}{T_s} v_s \qquad (15)$$

where v_{st} is the reading corrected to standard temperature. Therefore:

$$v_{st} = \frac{T_{st}}{T_s}\left(\frac{T_s - T_r}{T_r}\right)v' + \frac{T_{st}}{T_r}v_r \tag{16}$$

Consider the first term to the right of the equality sign. It may be reduced to relative insignificance in two ways, firstly by making v' sufficiently small through use of short tubing of very small bore, and secondly by controlling fluctuations in room temperature. Nevertheless, we must consider that the volumetric procedure again falls somewhat behind the manometric procedure insofar as dependence upon variables is concerned.

We compare the final relationships for both methods: v_{st} symbolizes the measurement of either h or v_r as the case may be, corrected to conditions of standard pressure and temperature.

The manometric method:

$$v_{st} = \frac{P_r - P_s}{P_{st}}V' + \frac{V'}{P_{st}}h \tag{5'}$$

The volumetric method:

$$v_{st} = \frac{T_{st}P_s}{T_sP_{st}}\left[\frac{P_r - P_s}{P_s}V' + \frac{T_s - T_r}{T_r}v' + \frac{P_rT_s}{P_sT_r}v_r\right] \tag{17}$$

Effects of Size of Fermentation Vessel

The manometric method.—As a starting point we may rewrite

$$v_{st} = \frac{h + P_r - P_s}{P_{st}}V' \tag{5}$$

Let us hold all conditions but V' constant and calculate what effect variation in V' will have upon a given v_{st} corresponding to n moles of carbon dioxide.

$$\Delta v_{st} = \frac{h + P_r - P_s}{P_{st}}\Delta V' \tag{18}$$

Dividing by v_{st} and multiplying by a hundred:

$$\frac{100\Delta v_{st}}{v_{st}} = \frac{100\Delta V'}{V'} \tag{19}$$

Or the percentage error induced in v_{st} by changing from one experimental system to another is equal to the percentage variation in V' in going from one to the other. We may conclude that the percentage variation in the V''s among a group of manometric systems should not exceed the percentage variations among a series of replicate determinations in any one of these systems. The restrictions laid down by this

rule are somewhat greater than are at first apparent because one must remember that

$$V' = V + v' - v^* \tag{6}$$

and that v^* has been defined as the non-gaseous volume of the fermenting dough. This definition may well be broadened somewhat to include the non-gaseous volume of any object or substance placed in the fermentation vessel. Therefore, if as is sometimes done, caustic soda or salt solution is placed within the vessel, it is important that the same volume of liquid always be used. Theoretically, the same volume of dough should be used, if consistent results are to be obtained. The criterion for the amount of care to be used in employing these considerations is again the percentage variation in V' as defined above that may be tolerated without causing errors in either h or v_{at} greater than are warranted by the reproducibility of the method.

It is now apparent that measurements obtained with different-sized doughs are not directly comparable; that is, readings obtained say with a 5-g. dough are not equal to half of the corresponding readings obtained with a 10-g. dough. Rigidly considered, all doughs studied by this method should be of identical non-gaseous volume.

Another source of error depending upon the bore of the manometer tube may be mentioned. This error is unimportant if the necessary precautions are taken. For any one manometric system, as h increases v' increases by an amount equal to

$$\Delta v' = \Delta V' = \frac{h\pi d^2}{4} \tag{20}$$

If for example one wishes to keep this error at less than 1% then

$$\frac{100\Delta V'}{V'} = 1 = \frac{100h_m\pi d^2}{4V'} \tag{21}$$

and

$$d < \sqrt{\frac{4V'}{100h_m\pi}} \tag{22}$$

$$d < 0.113\sqrt{\frac{V'}{h_m}} \tag{23}$$

If, for example, we take $V' = 200$ cc., and a maximum $h_m = 400$ mm. Then:

$$d < 2.53 \text{ mm.} \tag{24}$$

Or more generally, for any desired percentage error in V' or the corresponding percentage error in h or v_{at} for any maximum h_m and any V':

$$d < \sqrt{\frac{4V'}{100h_m\pi}\left(\frac{100\Delta h}{h}\right)} \tag{25}$$

where $\frac{100\Delta h}{h}$ is the permissible percentage error in h and h_m is the maximum value that h may be expected to attain.

The volumetric method.—Consider the analogue of equation 5:

$$v_{st} = \frac{T_{st}P_s}{T_sP_{st}}\left[\frac{P_r - P_s}{P_s}V' + \frac{T_s - T_r}{T_r}v' + \frac{P_rT_s}{P_sT_r}v_r\right] \qquad (17)$$

It is immediately apparent that errors induced by variations in either v' or V' are of a secondary nature; that is, neither v' nor V' is directly connected to the measured quantity v_r, but rather to the temperature and pressure corrections respectively. That is, this method is completely independent of vessel size in the absence of significant pressure and temperature corrections. When these are present, however, we may write for the dependence of v_{st} upon fluctuations in v' for any given set of conditions represented by T_s, T_{st}, T_r, P_s, P_{st}, P_r, and v_r:

$$\Delta v_{st} = \frac{T_{st}P_s}{T_sP_{st}}\left[\frac{T_s - T_r}{T_r}\right]\Delta v' \qquad (26)$$

Where Δv_{st} represents the variation in v_{st} induced by variations in v' of $\Delta v'$ among a group of measuring devices. With the realization that v' itself may be made relatively small by appropriate selection of short tubing of narrow bore, it is seen that $\Delta v'$ may be made to approach an insignificantly small magnitude. Certainly there is no technical difficulty in arranging to have $\Delta v'$ less than 1 cc.; this corresponds to about $2\frac{1}{4}$ inches of $\frac{3}{16}$ inch bore tubing. Further $|T_s - T_r|$ may be given a maximum value of say 10°C., and T_r a minimum value of 290°K. It is unlikely that P_s will exceed say 780 mm. Under these conditions of maximum dependence of v_{st} upon fluctuations in v', we may write

$$\Delta v_{st} = 0.036 \text{ cc.}$$

as the maximum deviation in v_{st} to be expected from this source.

We may now consider the dependence of v_{st} upon fluctuations in V'.

$$\Delta v_{st} = \frac{T_{st}}{T_s}\left(\frac{P_r - P_s}{P_{st}}\right)\Delta V' \qquad (27)$$

Now V' is limited in smallness by the mass of dough to which the equipment is adapted. It has been this writer's experience that V' may not be much smaller in cubic centimeters than some ten times the mass of the dough in grams. This ratio permits somewhat more than sufficient head room necessary for free expansion of the dough. Hence $\Delta V'$ among a group of volumetric systems may possess a significant magnitude.

We may assume, however, that with ordinary precautions $\Delta V'$ among a group of vessels may be kept below 5 cc. Further, it is unlikely that T_s would ever be smaller than 290°K, or that $|P_r - P_s|$ would exceed 5 mm. Hence we may calculate the deviation induced in v_{st} under the worst likely conditions:

$$\Delta v_{st} = 0.034 \text{ cc.}$$

We conclude that within the limits set for $\Delta v'$ and $\Delta V'$ one may expect v_{st} to be within 0.07 cc. of the correct volume, independent of the total volume of gas involved. This may be compared to the effects of similar variations of V' upon the v_{st} obtained via the manometric method:

$$\Delta v_{st} = \frac{h + 5}{760} 5$$

It is apparent that the variation in v_{st} is dependent upon the reading h. Even the relatively small value of $h = 100$ mm. induces an error in v_{st} of 0.691 cc.

Here at least the volumetric procedure possesses an advantage over the manometric. Less difficulty is involved in the construction of a group of replicate measuring systems. It may be argued, of course, that V' may be made so large that a variation in V' of 5 cc. may be considered without significance. Under these conditions, however, the size of dough must be increased proportionately if appreciable magnitudes of h are to be obtained. This involves other considerations such as the amount of gas confined within the dough mass and the difficulty in maintaining no more than, say, a 5 cc. difference in volume among a group of large vessels. A point of interest lies here. The study of gas production is basically the study of the number of moles of carbon dioxide produced; that is, the gas must be measured under conditions of known pressure and temperature. It is therefore advisable to arrange the determination so that the dough under consideration possesses as large a surface compared to its mass as is practical. This speaks for small doughs.

The smallness of the dough, however, is limited by the necessity of maintaining a dough large enough to be representative of the ingredients involved. The possibility of avoiding these difficulties by the use of suspensions rather than doughs is not to be given overly much attention, since it will be shown in a later paper of this series that gas production follows a radically different and less informative course under these conditions.

It may be well at this point to consider equipment actually in use. A manometric set-up on the market employs a metal fermentation

vessel, 261 cc. in volume. This vessel is threaded at its mouth. The cover, to which the manometer is attached, is screwed down upon a rubber gasket of some 2 mm. thickness. The error in V' due merely to difference in compression of 1 mm. is 3.97 cc. in 244 cc. (17 cc. deducted from V because of ingredients) or 1.6%. This corresponds to 1.6% error in either h or v_{st}.

We may attempt a more detailed study of this device and compare it to a similar study of the volumetric apparatus employed in this laboratory. We will assume minimum variations in V' for both types of systems of 5 cc. and minimum variations in v' for the volumetric systems of 1 cc. P_r, P_s, T_r, and T_s will be so chosen as to produce maximum likely errors; that is, $P_r = 780$ mm., $P_s = 775$ mm., $T_s = 300°$K., and $T_r = 290°$K. wherein it is assumed that $P_r - P_s > |5|$ mm. and $T_s - T_r > |10°|$ are unlikely possibilities. Table I presents the critical characteristics of the two types of instruments. The figures represent cubic centimeters.

TABLE I

	V	v'	v^*	V'	$\Delta v'$	ΔV
Manometric	261	—	17	244	–	5
Volumetric	83	15	8	90	1	5

Ten grams of flour are suggested for use in the manometric device; five grams of flour in the volumetric. We may calculate the deviation between the uncorrected and the corrected readings obtained via both types of instruments for the case in which $v_{st} = 100$ cc. and $v_{st} = 50$ cc. for the manometric and the volumetric systems respectively. For the former case we find $h_{\text{uncor.}} = 311.5$ mm. and $h_{\text{cor.}} = 300.1$. This corresponds to a 3.8% error in h for the manometric method. For the latter case we find $v_{\text{uncor.}} = 45.55$ cc. and $v_{\text{cor.}} = 50.00$ cc. This corresponds to a 8.9% error in v for the volumetric method. We may conclude that under the worst environmental and instrumental conditions, and if no corrections are attempted, the manometric equipment here under consideration will yield the more precise results.

Discussion

The advantage of some 5% in uncorrected precision of the manometric over the volumetric technique is offset by the following considerations: (1) The problem of preventing leakage in the manometric method under conditions of some two to three hundred millimeters of mercury pressure, compared to the practically zero pressure differential in the volumetric procedure. (2) The difficulty of determining whether or not the closed manometric system is or is not

completely sealed. One would have to force air into the system in order to check for such leakage, while with the volumetric procedure it suffices merely to lower the leveling bulb and note the constancy of reading. (3) The incidental but inherent distinction between fermentation under conditions of constant volume and variable pressure as compared to fermentation under conditions of constant pressure and variable volume. The latter technique simulates actual working conditions, and therefore permits use of gas production data obtained thereby in the interpretation of the baking test and in the study of fermentation kinetics; whereas it remains to be proven that gas production under variable pressure may be considered equivalent to gas production under constant pressure. The isolated note by Sandstedt and Blish (1936) barely can be considered supporting evidence. It can be shown that effects completely masked in plots of v_{st} against time become quite marked in plots of rates of gas production against time. (4) The greater fragility of manometric equipment. (5) The greater care required in maintenance of such equipment. Purity of mercury and cleanliness of manometer are relatively important.

One additional point is worthy of consideration. In some instances rates of gas production rather than total volumes of gas produced are of interest. The average rate of gas production $\bar{R}_{st}$ may be defined as the difference between v_{st2} and v_{st1}, where v_{st2} represents the corrected total volume of gas produced at some time indicated by the subscript 2 and v_{st1} represents the corrected total volume of gas produced at some earlier time represented by the subscript 1. This laboratory has found quarter-hour differences to be a rather close approach to corresponding instantaneous rates obtained by suitable extrapolation of one, half, quarter, and twelfth hour rates. Hence the units of $\bar{R}$ will be considered cubic centimeters per quarter hour per 10 or 5 g. of dough respectively for the manometric and volumetric procedures described above. Equations 5 and 17 may be rewritten:

$$\bar{R}_{st} = \frac{h_2 - h_1 + P_{r2} - P_{r1}}{P_{st}} V' \qquad (5'')$$

and

$$\bar{R}_{st} = \frac{T_{st}P_s}{T_sP_{st}}\left[\frac{P_{r2} - P_{r1}}{P_s} V' + T_s\left(\frac{T_{r1} - T_{r2}}{T_{r1}T_{r2}}\right)v' + \frac{T_s}{P_s}\left(\frac{P_{r2}}{T_{r2}}v_{r2} - \frac{P_{r1}}{T_{r1}}v_{r1}\right)\right] \qquad (17')$$

But it is extremely unlikely that $|T_{r1} - T_{r2}|$ will exceed 1°C. during the quarter hour of time involved. Further, it may be assumed that $|P_{r2} - P_{r1}|$ will not exceed 1 mm. during this interval. By the pro-

cedure employed in an earlier section of this paper we will calculate $(h_2 - h_1)$ corrected and uncorrected corresponding to a given R_{st} and will also calculate the corresponding uncorrected $(v_{r2} - v_{r1})$ for the volumetric case.

We will again assume values for the T's, P's, v' and V' such that maximum deviations between corrected and uncorrected values will be produced. For this purpose we select: $P_{r2} = 780$ mm.; $P_{r1} = 779$ mm.; $P_s = 775$ mm.; $T_{r1} = 291°$K.; $T_{r2} = 290'$K.; $T_s = 300°$K.; $v' = 16$ cc.; V' (manometric uncorrected) $= 244$ cc.; V' (manometric corrected) $= 249$ cc.; V' (volumetric) $= 95$ cc. $\bar{R}_{st}$ will be assumed 12.00 cc. and 6.00 cc. per quarter hour for the manometric and the volumetric methods respectively. v_{r2} will be assumed 40.00 cc. for the latter method. We find: $(h_2 - h_1)$ uncorrected $= 37.39$ mm. and $(h_2 - h_1)$ corrected $= 35.60$ mm. This corresponds to a 5.0% error in $\bar{R}_{st}$. For the volumetric method we find: $(v_{r2} - v_{r1})$ uncorrected $= 5.30$ cc. This corresponds to an error of 11.7% in $\bar{R}_{st}$, if no appropriate corrections are made. Yet the method in this laboratory has been found to yield replicate results in $\bar{R}$ with a maximum spread of no more than 2%–3%.

If one is content with this precision, then equation 17′ may be simplified as follows: Consider the term

$$T_s\left(\frac{T_{r1} - T_{r2}}{T_{r1}T_{r2}}\right)v'$$

Since the maximum divergence of all temperatures ordinarily does not exceed 5%, and since the term in question is merely a corrective term of only some 0.10 cc. under our conditions, we are justified in replacing T_s, and T_{r1} and T_{r2} of the denominator by T_{st}. The term

$$\frac{T_sP_{r2}}{P_sT_{r2}}v_{r2} - \frac{T_sP_{r1}}{P_sT_{r1}}v_{r1}$$

may be replaced by

$$\frac{T_sP_r}{P_sT_r}(v_{r2} - v_{r1})$$

since P_{r2} and T_{r2} are not expected to differ from P_{r1} and T_{r2} by more than 0.2% and 0.4% respectively and we may take $P_{r1} = P_{r2} = P_r$ and $T_{r2} = T_{r1} = T_r$. Equation (17′) now has the form

$$\bar{R}_{st} = \frac{T_{st}}{T_s}\left(\frac{P_{r2} - P_{r1}}{P_{st}}\right)V' + \frac{P_s}{P_{st}}\left(\frac{T_{r1} - T_{r2}}{T_{st}}\right)v' + \frac{T_{st}P_r}{T_rP_{st}}\bar{R}_r \quad (17'a)$$

But

$$\frac{T_{st}}{T_s}\left(\frac{P_{r2} - P_{r1}}{P_{st}}\right)V'$$

is merely a corrective term of at most some 0.20 cc. and since the maximum likely error introduced by letting $T_{st} = T_s$ is only 5%, the factor T_{st}/T_s may be omitted. By the same reasoning the factor P_s/P_{st} of the second term is avoided. Our simplified relationship for correcting rates of gas production to conditions of standard pressure and temperature finally takes the form:

$$\bar{R}_{st} = \frac{P_{r2} - P_{r1}}{P_{st}} V' + \frac{T_{r1} - T_{r2}}{T_{st}} v' + \frac{T_{st}P_r}{T_r P_{st}} \bar{R}_r \qquad (17'b)$$

where the subscript r designates a value midway between that designated by r_1 and r_2.

Within similar limitations, equation 17 appears as:

$$v_{st} = \frac{P_r - P_s}{P_{st}} V' + \frac{T_{r1} - T_{r2}}{T_{st}} v' + \frac{T_{st}P_r}{T_r P_{st}} v_r \qquad (17a)$$

Conclusions

Errors due to variations in barometric pressure, room temperature, and to variations in physical characteristics of replicate measuring systems may be of sufficient magnitude to warrant the application of corrective measures. If such corrections are not made, then the incidental data required to perform such operations should be recorded. The volumetric method is more sensitive to variations of pressure and temperature than is the manometric. The converse, however, is true for variation in the physical characteristics of replicate measuring devices. Finally, considering the manometric method, data recorded in units of pressure are lacking in physical significance unless the total internal volume available for free expansions of gas is recorded as well.

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GAS PRODUCTION IN YEAST FERMENTATION AND ITS APPLICATIONS. II. A VOLUMETRIC METHOD FOR THE STUDY OF GAS PRODUCTION

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Many chemical phenomena in liquid solutions are characterized by the liberation of a gaseous phase. The study of both the intensity and the extent of gas production has often led to information concerning the mechanism of the reaction. It is therefore not surprising that methods for the determination of gas production have received considerable study and development by many workers in many fields. The study of nitrosotriacetone-amine by Brönsted and King (1925), of yeast fermentation by St. John and Bailey (1929), Jørgensen (1931), Blish, Sandstedt, and Astleford (1932), and by Bailey (1939), and the determination of sugars via appropriate enzymes by Schultz and Landis (1932) constitute cases in point.

The problem of designing a method for the measurement of gas formation is then more a matter of adaptation of existing procedure rather than that of origination. This writer has shown (Part I of this series) that both of the alternative principles, measurement of volume under conditions of constant pressure, or measurement of pressure under conditions of constant volume, suffer relative advantages and disadvantages. Certain conditions were laid down for minimizing disturbing influences, such as fluctuations in temperatures and atmospheric pressure. Incidental advantages were ascribed to the volumetric technique, such as relative simplicity and strength of equipment, ease of maintaining essential cleanliness, and lower risk of leakage. A direct advantage lay in its measurement of gas production under conditions of constant pressure and the associated elimination of pressure as a variable—important or unimportant as it may prove to be. It is the purpose of this paper to describe the apparatus and the procedure which have in our hands during the past three years yielded data of relatively high precision concerning gas production in yeast-fermenting doughs.

Apparatus

Since in the procedure hereinafter to be discussed, 85.0 g. of dry flour (100.0 g. on a 15% moisture basis) are used, it is convenient to have a set of masses calibrated in 0.5% units of moisture such that a given mass represents that mass of flour of corresponding moisture content which is equivalent to 85.0 g. of said dry flour. The flour is

to be weighed into counterpoised pint Mason jars, the jars covered, and permitted to rest in the constant-temperature baking room until room temperature is reached; that is, the flour as well as all other ingredients is brought to 28°±1°C. before mixing, thereby avoiding temperature variability in the mixed dough. Balances used in weighing are such that all ingredients may be weighed to within 0.2% of the desired value. Water is delivered from a 100-ml. burette into 100-ml. Erlenmyer flasks, the flasks stoppered and stored until used. Yeast is weighed into Coors No. 000 evaporating dishes, covered with watch glasses, and also stored until used. This generally is at most an hour and a half. Storage for this period of time has, so far as we have been able to detect, no effect upon the subsequent behavior of the yeast. This agrees with the findings of Merritt, Blish, and Sandstedt (1932), with those of Cook and Malloch (1930), and with those of Sandstedt and Blish (1934). Mixing is carried out in a two-speed, double-unit Washburn Crosby mixer. Slow corresponds to 110 rpm. and fast to 220 rpm. Each unit possesses a hook mixing arm. The measuring systems and the fermentation chambers are described beneath their respective figures.

Procedure

All ingredients are weighed out in advance of mixing. One-hundred-gram samples of flour (15% moisture basis) are weighed directly into the Mason jars; 3.00-g. samples of yeast into the evaporating dishes; and distilled water to make 60% absorption (15% moisture basis) delivered from the 100-ml. burette into the Erlenmyer flasks. Supplementary ingredients are weighed on an analytical balance and, if soluble, are dissolved into their respective waters; if insoluble they are placed in individual evaporating dishes and incorporated into the dough in a fashion similar to that of the yeast. The "primitive formula" implied above was suggested to this writer some time ago by E. E. Werner.

The flours for runs No. 1 and No. 2 are placed each in one of the mixing bowls. On the quarter hour, Erlenmyer flask and evaporating dish No. 1 are uncovered. Some 10 ml. of the water is poured over the yeast. The mixture is stirred vigorously by means of a heavy pestle-like glass rod and transferred to mixing bowl No. 1. The dish is washed clear by three additional rinsings, after which the remaining liquid is poured into bowl No. 1. These operations should require one minute. Ingredients No. 2 are added to bowl No. 2 during the second minute prior to mixing. Mixing is begun two minutes after the quarter hour and consists of one minute on low and one minute on high. The dough temperature out of the mixer should be within 1° of 30°C., and the time is four minutes past the quarter hour.

Two 8.00-g. aliquots are taken from each dough and transferred to their respective vessels *A* (Fig. 2). These are attached to the measuring systems by means of the clamps *F* (Fig. 2) so that the aliquots from dough No. 1 go to burettes 1 and 2, while those from dough No. 2 go

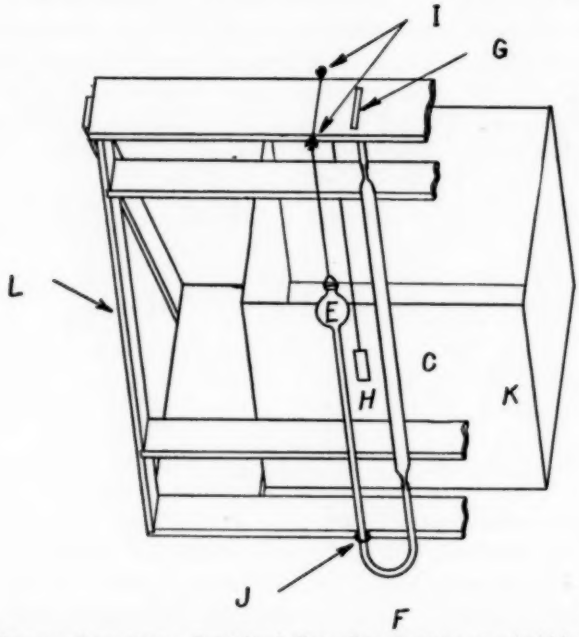

Fig. 1. The measuring system. Ten 100-ml. Hempel gas burettes, one of which, *C*, is illustrated above, are mounted on the wooden framework, *L*. Their lower outlets are connected via ¼-inch Neoprene tubing through eyes, *J*, to 100-ml. leveling bulbs, *E*. Tubing, burettes, and leveling bulbs are filled with paraffin oil. The latter are supported by braided strings which ride the pulleys, *I*, and are counterbalanced by the weights, *H*. The upper outlets of the burettes are connected respectively to the T-tubes, *G*, the front openings of which are subject to sealing and the rear openings of which are attached to the tubing connectors, *D*, of the fermentation chambers (Fig. 2) by means of $\frac{1}{16}$-inch Neoprene tubing. Total internal volume, *v'*, of the connections from top of *C* to *D* is some 14 ml. and is a constant common to the ten units. *K* is a constant-temperature water bath in which the fermentation chambers are immersed. It is equipped with an electrical stirrer, a 500-watt Cenco Knife heater, and a toluene-mercury thermoregulator. A thermionic relay avoids fouling at the mercury-platinum contact. Temperature is maintained at 30 ± 0.05°C. Lastly a brass framework is mounted within the bath. This supports the fermentation chambers by means of their extended brass plugs, *C* (Fig. 2).

to burettes 3 and 4. Precisely 13 minutes after the start of mixing (on the following quarter hour) system 1 is sealed by clamping the tubing attached to the front outlet of the T-tube, *G* (Fig. 1). Thirty seconds later, system 2 is sealed; similarly in half-minute intervals systems 3 and 4. Immediately after system 4 is sealed, ingredients No. 3 and No. 4 are transferred to their mixing bowls. Transferring should be completed and mixing begun on the nineteenth minute after the initial quarter hour. The aliquots for doughs No. 3 and No. 4 go to measuring systems 5, 6, 7, 8. Burette 1 is read to the nearest 0.05 ml. on the second quarter hour after mixing and in successive half-minute steps burettes 2, 3, and 4 are read, and burettes 5, 6, 7, and 8 are sealed.

Immediately thereafter the ingredients for the fifth dough are transferred to the mixer, and two minutes after the reading of burette 8, the last mix is started. And so on in half-minute steps first sealing,

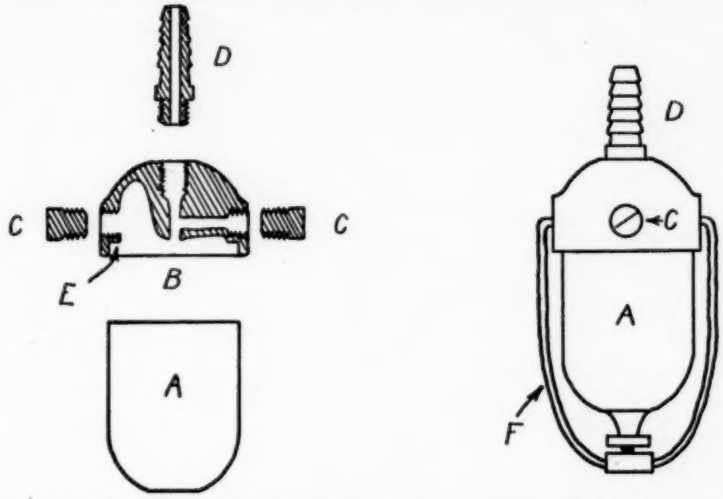

Fig. 2. Details of fermentation chamber: left, front cross section; right, side section view. These consist of reconstructed Tillotson Model OW-400A fuel strainers. *B* represents a cross section of the head. *E* is an inset ridge which serves as backing for a rubber gasket of 4-mm. thickness. The cork gasket supplied with the filter is unsatisfactory. *F* is a clamp which permits one to force the glass vessel *A* into close contact with the gasket. The internal volume of the fermentation chamber, *V*, is about 83 ml. The total internal volume from burette to and including chamber is then 97 ml. And since we will consistently employ an 8.00-g. aliquot of dough of volume v^0, assumed 8 ml., the total free volume of the system defined by $V' = V + v' - v^0$ is equal to 89 ml.

then subsequent quarter hourly readings of each of the measuring systems are made. Hence, the volume of gas produced as a function of time is obtained.

Significance of Rate of Gas Production [1]

It may prove worth while to go into somewhat greater detail concerning the definition and physical significance of the rate of gas production. More precisely, it should be called the time rate of gas production. By this is meant the volume of gas produced in a given

[1] The cereal literature often speaks of "gas production," "rate of gas production," "gas retention," and "gassing power." Unfortunately, these terms have been used somewhat loosely, and the word "rate" has often proxied for "volume" and both have been supplanted on occasion by "power." The unit of volume is generally the cubic centimeter, or perhaps better, the milliliter. We therefore define "gas production" as the number of milliliters of carbon dioxide, corrected to standard temperature (30°C.) and pressure (760 mm. of Hg) that is produced from time of mixing to time of reading under standard conditions of fermentation. The use of "gassing power" is neither quite correct in implied units, nor quite fortunate in its visual similarity to horsepower. Properly, were one interested in measuring power of fermentation, one would determine the rate of gas production say in milliliters per minute and multiply this result by the atmospheric pressure say in dynes per square centimeter. It remains, therefore, that rate rather than volume of gas produced is a measure of power, though even this is not power itself. "Gassing power" seems like a pretender to the position of flour characteristic. It may appear that these distinctions are somewhat academic in the present instance and that the matter of constant factors may well be neglected. One point, however, remains as a significant detail. That which usually is determined as "gassing power" is an average rate and hence a measure of an average power. "Gas retention" apparently implies the maximum volume attainable by a dough under fixed conditions of fermentation. See St. John and Bailey (1929), Working (1929), and Bailey (1939). More will be said about this at another time.

unit of time. For practically all chemical and physical phenomena this function varies continuously with time; that is, if it is plotted against time it yields a smooth curve without discontinuities. Mathematically, it may be represented by the change in volume of gas produced divided by the change in time from one measurement of volume to the other. Symbolically it is represented by

$$\bar{R} = \frac{\Delta v}{\Delta t}. \tag{1}$$

Here $\bar{R}$ represents the average rate of gas production for the time interval Δt. Δv represents the change in volume during the interval Δt. If the interval of time between the two measurements of volume is decreased till it approaches zero, then the infinitesimal amount of gas produced divided by the infinitesimal period of time yields the instantaneous rate of gas production at a time lying somewhere within the infinitesimal time interval of measurement. That is, the instantaneous time rate of gas production is associated with and is a function of a perfectly defined instant of time. Symbolically this may be represented by

$$R = \frac{dv}{dt}. \tag{2}$$

It is this quantity, R, that possesses mathematical significance but which unfortunately is not yet subject to direct measurement.[2] It can, however, be approached by $\bar{R}$, the average time rate of gas production. R is subject to far simpler theoretical treatment than v, and once obtained as a function of time can be converted to v by ordinary mathematical techniques of integration. Once v is obtained as a function of time, obviously $\bar{R}$ is obtained as well. The significance of R may be represented simply by the following expression

$$R = \frac{dv}{dt} = KZ_tG_t + K'Z_t'F_t, \tag{3}$$

where K is the so-called velocity constant for glucose fermentation,
K' is the so-called velocity constant for fructose fermentation,
Z_t is the concentration of zymase effective in glucose fermentation at time t,
Z_t' is the concentration of zymase effective in fructose fermentation at time t,
G_t is the concentration of glucose at time t, and
F_t is the concentration of fructose at time t.

[2] Note the ingenious attempt by James and Huber, Cereal Chem. 5: 181–182 (1928). Water displaced by gas production enters a cylinder at the base of which is a small orifice. The rate of gas production (rate of inflow of water), R, is measured by the height of water, h, in the cylinder. It is evident that $dh/dt = C(R - r)$ where C is the ratio of h to volume of water in cylinder and r represents the rate of leakage through orifice. But $r = f(h)$. Therefore $R = 1/C\ dh/dt + f(h)$ and is not measured by h itself. An uncertainty of order $1/C\ dh/dt$ is involved. This is equivalent in practice to the averaging of R over a finite time interval, Δt.

It is understood that temperature and pressure are held constant. Also implicitly, it is assumed that either only hexoses are fermentable or that the higher saccharides, by whatever mechanism they may, must pass through the hexose stage. Nothing further can be done with this until Z_t, Z_t', G_t and F_t are evaluated as functions of time. The problem is not necessarily impossible of solution and if solved would permit of definite understanding of the effects of those ingredients which influence the rate of gas production.

It has been suggested that the instantaneous time rate of gas production is not subject to direct measurement but that it may be approached as closely as we please (within the limits of our instruments) by making the time intervals between measurements as small as possible, or at least so small that further decreases in the time interval do not affect the results obtained. Therefore as is evident from our procedure, the difference in volume between any two consecutive readings of a given burette may be considered the average rate of gas production in milliliters per quarter hour per 8 g. of dough at the average time lying midway between the two actual times at which the necessary readings were made.

Our practice, when plotting rates versus time, has been to associate a given rate with the second of the two times involved. This simplifies recording and plotting of data, and if done consistently in no manner affects the relative interpretation of the plots; it is equivalent to shifting the entire curve $7\frac{1}{2}$ minutes in the direction of greater time. Hence if one wishes to compare rates obtained with Δt of say 5, 15, and 45 minutes, he must be certain to select average rates obtained at corresponding average times. For example, consider Tables I and II, in which typical data are recorded, readings having been taken at five-minute intervals rather than at our usual quarter-hour intervals. These rates, corrected to similar units, are plotted against their corresponding average times in Figure 3. We wish to indicate that the difference between the five-minute and the quarter-hour rate curves is no greater than the experimental error involved in the determination of the five-minute rates, and we are therefore justified in at least tentatively considering quarter-hour rates as a satisfactory approach to instantaneous rates. Hence, through such measurements, we may attempt to study the functional relationship, equation 3. Further, a glance at Tables I and II or study of the procedure used in obtaining these data, is sufficient to indicate a certain lack of absolute definition in the burette readings of volume; that is, there is an unknown and very likely variable quantity of gas formed during the first quarter-hour. Its magnitude may be established by extra-polation of either the volume or rate-versus-time curve to zero time. Mere observation

TABLE I

AVERAGE RATE OF GAS PRODUCTION (Δt = 5 MINUTES) AS A FUNCTION OF TIME

Minutes [1]	Volume (*ml.*)	5-min. rate [2] (*ml./5'*)	5-min. rate [3] (*ml./15'*)
0	Mixing begun	——	—
5	—	——	—
10	—	——	—
15	0.00	System sealed	—
20	0.95	0.95	—
25	2.00	1.05 (22½')	3.15
30	3.10	1.10	—
35	4.45	1.35	—
40	5.95	1.50 (37½')	4.50
45	7.55	1.60	—
50	9.15	1.60	—
55	10.80	1.65 (52½')	4.95
60	12.40	1.60	—
65	13.90	1.50	—
70	15.35	1.45 (67½')	4.35
75	16.70	1.35	—
80	18.10	1.40	—
85	19.45	1.35 (82½')	4.05
90	20.85	1.40	—
95	22.25	1.40	—
100	23.80	1.55 (97½')	4.65
105	25.45	1.65	—

[1] Time from start of mixing, the average one minute shifted, mentioned at end of section on consistent errors, being neglected.
[2] Figures in parenthesis are the average times of which the average rates are a function.
[3] Rates of preceding column multiplied by three.

TABLE II

AVERAGE RATES OF GAS PRODUCTION (Δt = 15 AND 45 MINUTES RESPECTIVELY) AS FUNCTIONS OF TIME

Minutes	15-min. rate (*ml./15'*)	45-min. rate (*ml./45'*)	45-min. rate [1] (*ml./15'*)
0	Mixing begun	——	—
15	System sealed	——	—
30	3.10 (22½')	——	—
45	4.45 (37½')	——	—
60	4.85 (52½')	12.40 (37½')	4.15
75	4.30 (67½')	13.60 (52½')	4.55
90	4.15 (82½')	13.30 (67½')	4.45
105	4.60 (97½')	13.05 (82½')	4.35

[1] Rates of preceding column divided by three.

of such graphs will show the relatively higher precision of rate extrapolations. This is at least one advantage in using rate- rather than volume-versus-time curves as a means of recording data, both for one's own use and for publication purposes. A second overwhelming advantage may also be mentioned at this time. The rate-versus-time curve is roughly some ten times as compact; that is, for a given amount of available space one may plot to an additional significant figure.

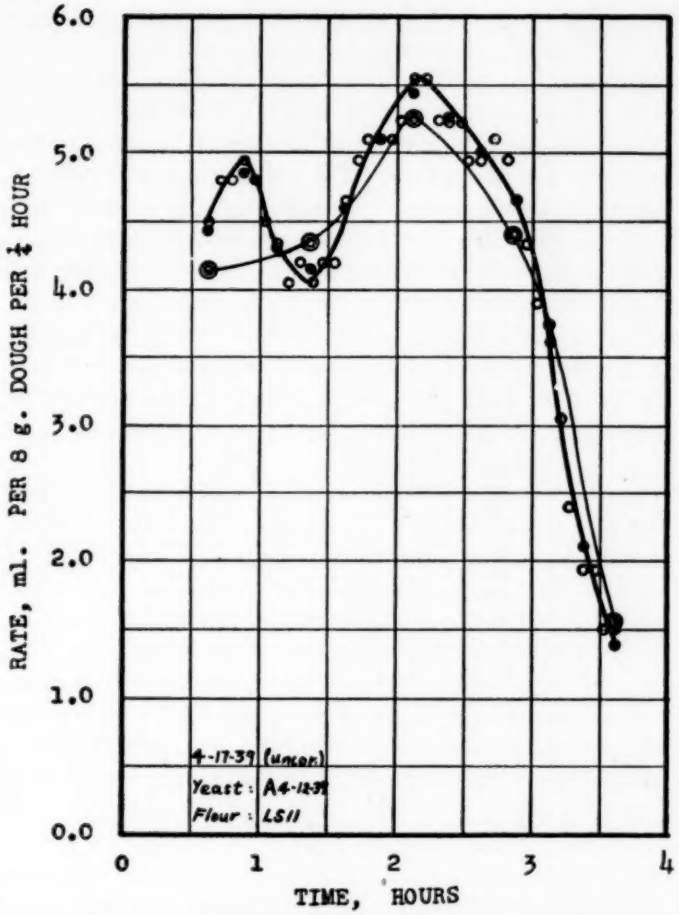

Fig. 3. Variation of average rate of gas production with variation of interval between measurements of volume. Open circles, 5-minute intervals; closed circles, 15-minute intervals; double circles, 45-minute intervals.

Consistent Errors

At the risk of appearing somewhat overcritical, we may now consider a source of barely tangible consistent error introduced by the procedure. There is a degree of uncertainty concerning the zero time —that is, concerning the instant at which fermentation starts. We may arbitrarily consider this time to be synonymous with the moment mixing begins.

By so doing, burette 1 is sealed 13 minutes after, while burette 4 is sealed 14½ minutes after the start of fermentation. Hence, an error due to this 1½-minute shift along the time axis may be involved when results obtained via random measuring systems are indiscriminately compared. The magnitude of this error is not sufficiently large, at

least for our present purposes, to warrant corrective measures in procedure. Its order, however, may be estimated as follows: The maximum rate of gas production as observed under our conditions is some 6.5 ml. per quarter, per 8 g. of dough. Thus, since the maximum error in total volume of gas produced occurs at the time of maximum rate, this error must be 0.65 ml. The volume of gas formed up to this time is generally some 30 ml. Therefore the maximum percentage error in volume from this source may be placed at about 2%.

In practice, however, one is not usually interested in the volume of gas produced from zero time to time of maximum rate; rather, one is interested in the total volume of gas produced up to the time at which the yeast becomes relatively exhausted. At this time, the rate of gas production is about 1 ml. per quarter hour and the total volume of gas produced is some 50 mls. Hence, the 1½-minute shift in time results in only a 0.2% error in volume.

One may likewise consider the error introduced in the measurement of the rate of gas production. The controlling factor is the acceleration—or change in rate. Normally this is not greater than 2 ml. in absolute magnitude per quarter hour per quarter hour. At the time of maximum acceleration the rate generally lies between 3 and 5 ml. per quarter hour. Therefore the maximum error in the rate of gas production due to the source under consideration may not be expected to exceed 0.20 ml. per quarter hour, or some 4%–7%. As is evident this magnitude of error occurs only at inflection points on the rate-versus-time relationship. The error is practically nil at maxima and at minima. Furthermore, the additional half-minute shift along the time axis involved in assuming that the time of sealing follows mixing by a quarter hour rather than by 14½ minutes may be neglected. We may, however, with an average error in time no greater than one minute assume, if the need arises, that all burettes are sealed on the average 14 minutes after their respective starts of mixing; that is, we can therefore cut the maximum time shift from 2 minutes to 1 minute.

General Discussion

The first of the essential requirements to be fulfilled by measurements of any kind is that of reproducibility. Figure 4 represents a group of seven individual determinations of rate as a function of time upon the same ingredients under identical conditions, and upon the same day. The width of the curve, or band, indicates the maximum spread among measurements, and denotes the precision of which the measuring system and procedure are capable. The average volume of gas produced in 3¾ hours was 54.60 ± 0.26 ml. and the maximum deviation of any one determination of volume from this average was

0.45 ml. The average percentage deviation from the average is then 0.48%. This does not compare unfavorably to many analytical procedures. The average value of the rate of gas production at the second

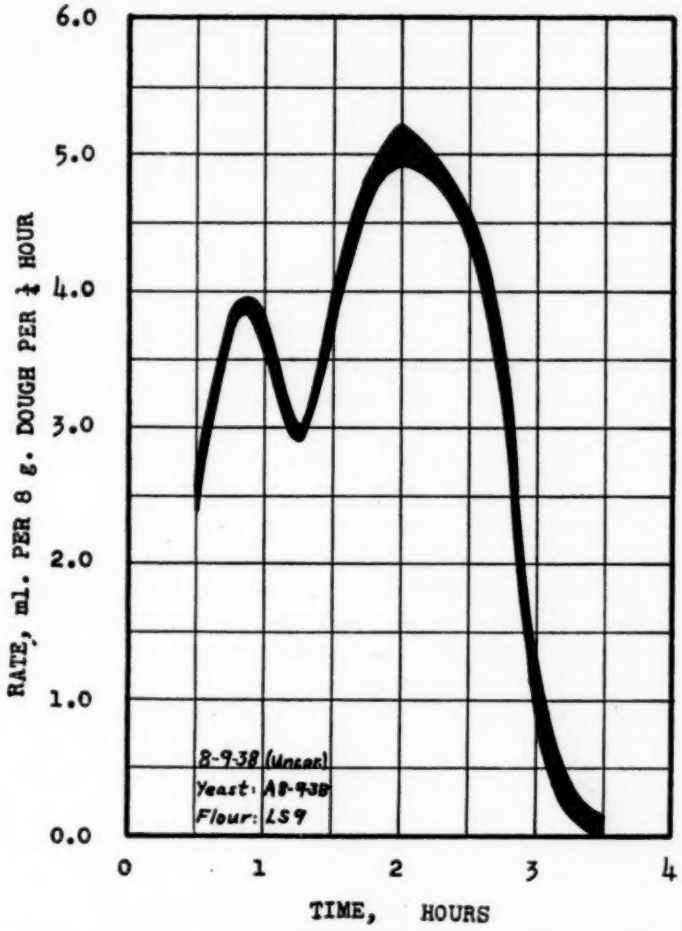

Fig. 4. Seven replicate determinations of rate of gas production as a function of time. All points are displaced downward 1.0 ml.

maximum is 5.98 ± 0.08 ml. per quarter hour, and the greatest deviation from this value was 0.22 ml. in one case and 0.08 ml. or less in all others as can be seen from Table III. This corresponds to an average percentage deviation from the average of some 1.3%, which is about as good as can be expected with the present measuring systems, since the burettes are calibrated in 0.2-ml. units and estimations to 0.05 ml. cannot be considered certain.

TABLE III

SEVEN REPLICATE DETERMINATIONS OF RATE AT SECOND MAXIMUM AND 3¾-HOUR GAS PRODUCTION AS INDICATION OF REPRODUCIBILITY OF METHOD

Rate at 2nd. peak (*ml./15'*)	Deviation from average	Volume at 3¾ hrs. (*ml.*)	Deviation from average
6.20	0.22	54.70	0.10
6.05	0.07	54.30	0.30
5.90	0.08	54.75	0.15
5.95	0.03	54.20	0.40
5.90	0.08	54.40	0.20
5.90	0.08	55.05	0.45
5.95	0.03	54.80	0.20
Average 5.98	±0.08	54.60	±0.26
or —	±1.3%	—	±0.48%

It is common practice in analytical methods, particularly when concerning reagents of questionable purity, to lay down certain specifications for such reagents, and since the procedure as outlined above possesses at least certain characteristics in common with analytical procedures, it is well to attempt some sort of standardization of the essential reagents in this case as well. Three basic reagents are involved: flour, water, and yeast.

It is obvious that for the study of yeast a standardized or standard flour is required, while for the study of flour a standardized or standard yeast is necessary. Unfortunately standardized ingredients of this type are not yet available. A standard flour, however, may be considered any flour which does not suffer changes in its rate of gas-production characteristics with period of storage. We find that flour stored at 2°–4°C. in sealed cans appears to fulfill this requirement for a period of more than a year. This cannot be definitely ascertained, however, since our only means of checking rate characteristics depends upon uniformity of yeast. Random variations from time to time in rate data of a standard flour stored under these conditions are taken by us to indicate yeast rather than flour variability. If this is true, as it appears to be, it seems possible that a group such as the cereal chemists might make some attempt to set certain rate specifications for a standard flour. Such a flour could be prepared by suitable blending of other flours, and could be packed and stored and sold just as are other analytical reagents.

Yeast is a somewhat more difficult problem. In Table IV are presented random data obtained with our standard flour over a period of some eight months. Several comments are in order. Firstly, there appears to be no consistent trend of data with time. Secondly, yeast is subject to variations in at least three major respects, maltase content, invertase content, and zymase content—these terms being used in a

TABLE IV

RATES AT MAXIMA AND FOUR-HOUR GAS PRODUCTION CORRECTED TO 30°C. AND 760 MM. OF HG OBTAINED OVER EIGHT-MONTHS PERIOD VIA FLOUR STORED IN SEALED CANS AT 2°–4°C. AND RANDOM SAMPLES OF YEAST A

Date	Rate at 1st. peak (*ml./15'*)		Rate at 2nd. peak (*ml./15'*)		Volume, 4 hrs. (*ml.*)	
12/18/39	5.00	0.00	5.40	0.12	58.40	0.72
12/13/39	4.70	0.30	5.30	0.22	58.45	0.77
11/30/39	5.05	0.05	5.50	0.02	59.55	1.87
11/27/39	5.15	0.15	5.50	0.02	57.80	0.12
11/16/39	4.90	0.10	5.60	0.08	57.80	0.12
11/15/39	4.70	0.30	5.50	0.02	57.20	0.48
11/10/39	5.50	0.50	5.85	0.33	62.15	4.47
10/30/39	5.10	0.10	5.70	0.18	58.40	0.32
10/17/39	5.90	0.10	5.35	0.17	57.65	0.03
10/ 5/39	4.85	0.15	5.40	0.12	55.00	2.68
10/ 2/39	5.00	0.00	5.50	0.02	55.60	2.08
9/29/39	4.80	0.20	5.15	0.37	54.80	2.88
9/27/39	5.15	0.15	5.40	0.12	55.20	2.48
8/28/39	5.10	0.10	5.55	0.03	56.40	1.28
7/21/39	4.90	0.10	5.55	0.03	56.00	1.68
6/26/39	5.10	0.10	5.70	0.18	55.95	1.73
6/ 6/39	4.95	0.05	5.65	0.13	57.70	0.02
5/ 5/39	5.10	0.10	5.80	0.28	59.65	1.97
4/13/39	5.15	0.15	5.55	0.03	60.45	2.77
4/11/39	4.80	0.20	5.40	0.12	59.35	1.67
Average	5.00	—	5.52	—	57.68	—
Average deviation from average	—	0.15	—	0.13	—	1.51
Percentage deviation	—	3.0%	—	2.4%	—	2.6%

sense broad enough to include any associated co-enzymes and the like. Other variable characteristics of yeast such as permeability of cell membranes undoubtedly play a role. Hence an indefinite number, certainly greater than three, of points on the rate-versus-time curve is needed to characterize the yeast; in fact a number of points sufficient to solve the unknown functional relationship between rate and time for all its parameters is required. Certain points on the curve are subject to smaller errors in measurement and correspond to a simplified form of the unknown functional relationship. These points occur at maxima and at minima. An additional point may be taken as the total volume of gas produced up to some arbitrary time at which the rate becomes so low that further increments of volume may be considered completely a flour rather than a yeast characteristic. Hence in Table IV we tentatively report rates at the first and second maxima, and volume of carbon dioxide produced in four hours. In doing so, we make the rather crude hypothesis that any two yeasts operating on a standard flour and yielding rates-of-gas-production-versus-time data such that the maxima and the four-hour gas production of the

one yeast correspond to those of the other, then the complete curve of the one is congruent with that of the other. Thirdly, the average percentage deviation from the average of rates at second maxima for data obtained over an eight-month period (Table IV) with different yeast samples of the same brand is some twice as great as the corresponding percentage deviation for data obtained on the same day with a given yeast sample (Table III). Further, the average percentage deviation from the average of four-hour volumes under the former conditions is some five times as great as for the latter conditions. Fourthly, it is our experience that variations in yeast of the order indicated in Table IV are sufficient to influence results obtained with the A.A.C.C. baking test. Fifthly, these findings concerning yeast variability are in agreement with those of Cook and Malloch (1930) but are in disagreement with those of Sandstedt and Blish (1934) and with those of Bohn and Favor (1939).

The problem of yeast standardization thus resolves itself into several distinct problems. Figure 5 serves to classify three different brands of yeast. It is to be noted that yeasts *A* and *C* both exhibit double maxima while yeast *B* displays only a tendency in this direction. The existence of two maxima under certain conditions has been reported by Lamour and Bergsteinsson (1936). They present evidence to prove that the two peaks merge into one under conditions of either sucrose excess or sucrose absence. Differences in magnitude and position of peaks obviously are related to difference in yeast characteristics. These differences may be associated with a great many causes by those interested in hypothesizing. Our own attempts we will reserve for a while longer. In reference to the three types of yeast here designated as *A*, *B* and *C*, it is our understanding that the differences indicated by Figure 5 are more likely attributable to conditions of growth and propagation rather than to any inherent differences of strain. If this is true, we may conclude that the manufacturers of yeast, though not having succeeded in producing an ingredient of reagent-like uniformity, have nonetheless performed a remarkable feat. The three types *A*, *B* and *C* show variations from time to time of order similar to that suggested by Table IV.

All data reported in this paper and all data to be presented in the future unless otherwise restricted, will be obtained with yeast of type *A*. Our selection of yeast *A* is in no manner associated with commercial utility; rather it is associated with somewhat greater general availability; and, more important, the magnitude and position of its peaks permit of sharper differentiation of flour characteristics.

The first part of the problem of yeast standardization is therefore the selection of type. Further, it is insufficient merely to typify the

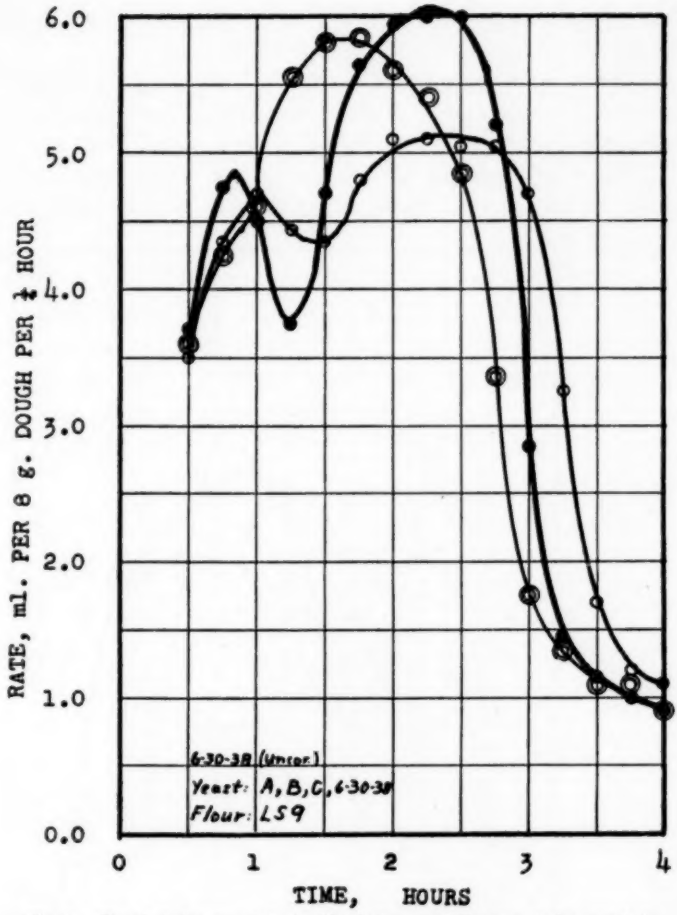

Fig. 5. Closed circles, yeast *A*; double circles, yeast *B*; open circles, yeast *C*.

yeast as most workers have done and are doing as *A*, *B*, *C*, *X* or *Y* unless characteristics such as rate-versus-time data obtained under standard conditions are recorded as well. A few isolated workers have taken the liberty of referring to yeast by brand name; others have very likely been hesitant to do so. It is evident as suggested by Eva, Geddes, and Frisell (1937) and by Bohn and Favor (1939) that a step such as here indicated must be taken before any agreement of data obtained by different workers may be expected. This writer is aware of the fact that the differences in type suggested by Figure 4 are reduced perhaps sometimes even to the vanishing point when the nutrient medium (the dough) is cluttered up with large quantities of saccharogenic material. But the differences in type, incipient or otherwise, nevertheless remain and in work of a scientific nature must

be indicated just as we unhesitatingly say phenylhydrazine by Eastman, or synthetic rubber tubing by Dupont.

Once the type is selected, the second part of the problem, the maintenance of uniformity within the type, must be approached. This unfortunately has not been done successfully as yet so far as we know. That it can be done we are certain—and in a practical fashion. One attack is at present under investigation in this laboratory. It

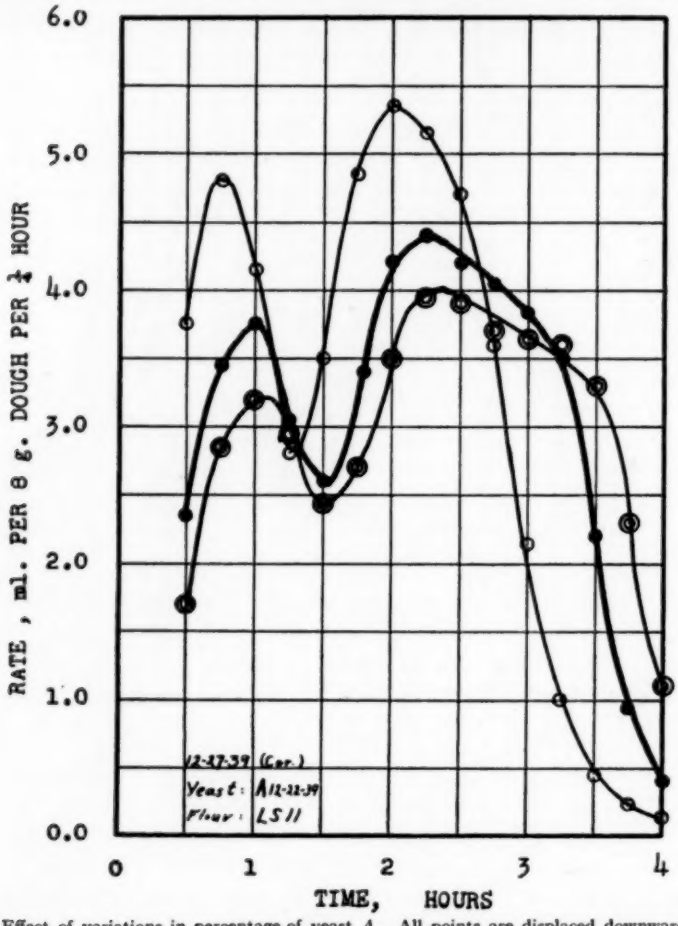

Fig. 6. Effect of variations in percentage of yeast *A*. All points are displaced downward 1.0 ml. Double circles, 2.6%; closed circles, 3.0%; open circles, 4.2%.

is based on the hypothesis that the ratio of the active constituents of random samples of yeast of given type is more constant than the actual quantity of these constituents. It is based further on the fact that any of the yeasts under consideration can be stored at some 4°C. for at least ten days without tangible changes in rate characteristics. We are

therefore studying the effects of variation in percentage yeast upon first and second maxima, and upon the volume of gas produced in four hours. This work is being done with our standard flour. Figure 6 represents the rate, corrected to standard temperature and pressure, as a function of time for 2.6%, 3.0%, and 4.2% yeast. The volumes of gas produced in four hours are 59.45, 60.55, 61.15 ml. respectively. These present the difficulty of refuting to some extent our hypothesis; that is, the variation in four-hour volume as indicated in Table IV cannot be attributed to only variation in effective yeast concentration, but must be attributed rather to the possible random presence of sugar in the yeast, variable glycogen storage capacity (see Sandstedt and Blish, 1939), possible variations in ratio of active constituents, or to an experimental error greater than we think likely. At any rate, our hypothesis may still hold sufficiently to permit standardization of yeast as to peak characteristics.

Table V presents the critical data. These may be described within

TABLE V

EXPERIMENTAL AND CALCULATED RATES AT MAXIMA (ML./15') AND FOUR-HOUR GAS PRODUCTION (ML.) CORRECTED TO 30°C., AND 760 MM. OF HG AS FUNCTIONS OF YEAST CONCENTRATION

Y	M_1 (exp.)	M_1 (calc.)	M_2 (exp.)	M_2 (calc.)	Volume (4 hrs.)
2.60	4.20	4.34	4.95	5.05	59.45
2.70	4.40	4.46	5.10	5.13	59.80
2.85	4.70	4.57	5.30	5.25	60.20
3.00	4.80	4.70	5.40	5.37	60.55
3.30	4.95	4.97	5.60	5.62	60.05
3.80	5.30	5.42	6.05	6.03	60.85
4.20	5.80	5.78	6.35	6.37	61.15

the range of yeast variation under consideration by the following relationships:

$$M_1 = 0.900\ Y + 2.00, \tag{4}$$

$$M_2 = 0.825\ Y + 2.90, \tag{5}$$

where M_1 and M_2 respectively refer to the rates at first and second maxima. Y represents the percentage of yeast based on flour. With these relationships established once and for all for a given standard flour, we propose to accept $M_1 = 5.00$ and $M_2 = 5.50$ as yeast specifications for the standardization of yeast as determined in our standard flour.

We further propose to obtain a week's supply of yeast say each Friday. On Friday or Saturday we will determine the rate of gas production for this yeast as a function of time in our standard flour and in the manner described in the procedure. We will then insert the

values of the first and second maxima into equations 4 and 5 respectively and calculate the corresponding Y for both. The average of these figures, $\overline{Y}$, will then be taken as measuring the effective concentration of the 3% of yeast actually used. That is

$$\overline{Y} = 0.555\ M_1 + 0.606\ M_2 - 2.87. \tag{6}$$

We will then compute the quantity of yeast necessary to satisfy our specifications and will employ this quantity of the yeast in all the following week's work—and so on.

For the yeast used in obtaining the data in Table V, 3.24% is required to meet specifications. Thus for future samples of yeast, we will employ 3.00% on the preliminary standardization test and will by means of equation 6 compute the average effective concentration $\overline{Y}$ corresponding to the observed peaks. Then by means of

$$Y' = \frac{(3.24)(3.00)}{\overline{Y}} \tag{7}$$

we will calculate Y' as the percentage of the new yeast to be used in order to maintain specifications. The results of this study will be reported upon compilation of sufficient data. The attack described is presented at this time merely as an example of what may at least be attempted in the standardization of yeast.

The third reagent required in the procedure is water. It seems self-evident to this writer that there is little choice other than the selection of distilled water.

Summary

A refined volumetric method for the determination of gas production and a practical procedure to be used in such measurements are described in detail. Certain sources of error in these measurements and possible corrective measures are described. A tentative attack on the problem of yeast standardization is offered.

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A METHOD FOR THE DETERMINATION OF NONPROTEIN NITROGEN IN SOYBEAN MEAL

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There is no sharp, clear-cut line of demarcation between proteins and the nitrogenous nonproteins, and the more or less arbitrary analytical differentiation between them depends on the method used. Phosphotungstic and trichloracetic acids are probably used as widely as any of the protein precipitants, and they also represent the two extremes with respect to precipitating power. Trichloracetic acid has been used in concentrations ranging from 1% to 10%, apparently without much attention to the effect of the different acid concentrations. Cristol and Monnier (1936) state that, with blood plasma, a solution containing a final trichloracetic acid concentration of 2.4% (Lefaux's method) leaves an appreciable amount of protein unprecipitated, an acid concentration of 2.7% (Goiffon and Spaey's method) leaves less protein unprecipitated, while a solution with 10% acid concentration (Moog's method) is protein-free. Hiller and Van Slyke (1922) state that trichloracetic acid in concentrations of 5% or less does

[1] A cooperative organization participated in by the Bureaus of Agricultural Chemistry and Engineering and Plant Industry of the U. S. Department of Agriculture, and the Agricultural Experiment Stations of the North Central States of Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin.

not precipitate peptones or any of the smaller protein fragments, whereas phosphotungstic acid precipitates peptones, polypeptides, and some of the amino acids. Thomas (1927), working with apple leaves and twigs, found that the precipitation of proteins from a hot aqueous extract with acetic acid, trichloracetic acid, and colloidal ferric hydroxide gave comparable results. Copper hydroxide gave results which were quite different, because it precipitated polypeptides and some amino acids along with the protein.

Trichloracetic acid was suggested as a protein precipitant by Greenwald (1915) 25 years ago. It is exceedingly soluble in water, and any excess is easily removed by decomposition into chloroform and carbon dioxide in hot solution. Polypeptides are not precipitated by it and proteins are not hydrolyzed even by a large excess of moderately concentrated acid.

Proposed Method

The method herein proposed was evolved from the experimental work which is presented later in the paper. After investigating the effect of the acid concentration, the particle size and oil content of the meal, and the variety of the bean upon the values obtained for nonprotein nitrogen, the following method is proposed for its determination:

A 1-g. sample of meal is weighed into a 200-ml. centrifuge bottle and extracted with 40 ml. of 0.8*N* (13.6%) trichloracetic acid for ½ hour in a mechanical shaker. The suspension is centrifuged for 5 to 6 minutes at a maximum R.C.F. of 1,975 times gravity and a 25-ml. aliquot of the clear supernatant liquid is used for a nitrogen determination by the Kjeldahl-Gunning-Arnold method. Fat-free meal is preferable but not necessary if the oil content is known for purposes of calculation or if the results are to be expressed in terms of the whole bean. The meal should be no coarser than that produced by grinding through the 1-mm. screen of a Wiley mill. The screen analysis of a fat-free meal ground in this manner is as follows: 13.4% is retained on the 35-mesh screen, 47.2% on the 60-mesh, 14.0% on the 80-mesh, 4.7% on the 100-mesh, and 20.5% passes through the 100-mesh screen.

Effect of Acid Concentration

The beans used in the first part of this work were of the Dunfield variety grown at Lafayette, Indiana, during 1937. They were flaked and extracted with petroleum ether (boiling range 35°–60°C.) in a modified Soxhlet extractor until free from fat. The flakes were then ground in a ball mill to pass through a 100-mesh sieve. The moisture content of this meal was 9.50%, and the nitrogen content, 7.75%.

Smith and Circle (1938) extracted fat-free soybean meal with trichloracetic acid and found that the minimum nitrogen extraction at the

isoelectric point (pH 4.1) was followed by a rise and then a second minimum as the acid concentration was increased. More concentrated acid ranges were investigated by the extraction technique described in a publication by Nagel, Becker, and Milner (1938). A $2\frac{1}{2}$-g. sample of fat-free meal was extracted for $\frac{1}{2}$ hour with 100 ml. of solvent and the entire sample used for a nitrogen determination. The results of these extractions are given in Table I and represented

TABLE I

NITROGEN EXTRACTED[1] BY TRICHLORACETIC ACID OF DIFFERENT CONCENTRATIONS

Acid normality	Milligrams of nitrogen extracted per gram of meal
0.2	5.01
0.3	3.94
0.5	3.36
0.65	3.14
0.75	3.27
0.85	3.16
1.0	3.19
1.2	3.31
1.7	4.11
4.0	73.9

[1] Total nitrogen present was 77.8 mg. per gram of meal.

graphically in Figure 1. The first portion of the curve in Figure 1 is from the data of Smith and Circle and is reproduced here to show the

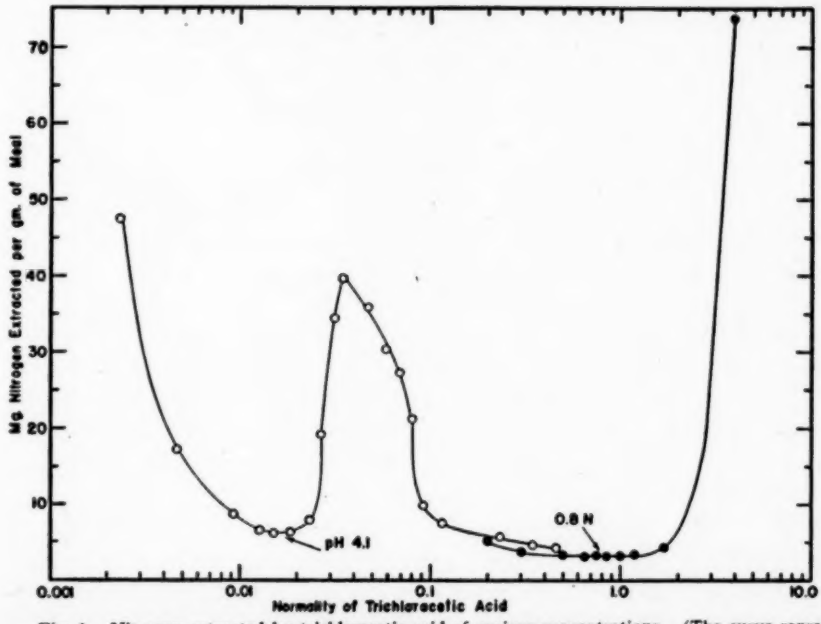

Fig. 1. Nitrogen extracted by trichloracetic acid of various concentrations. (The curve represented by open circles is from the data of Smith and Circle. The scale of the abscissa is in logarithmic units. Total nitrogen present was 77.8 mg.)

course of the extractions over the entire acid range. The two curves do not agree exactly because two different bean samples were used. The beans used by Smith and Circle were of the Illini variety and contained 7.80% nitrogen and 9.28% moisture. The 0.8*N* acid extraction lies approximately in the center of the extraction minimum extending from 0.65*N* to 1.0*N* acid, so 0.8*N* trichloracetic acid was chosen as the extracting agent.

The possibility of the adsorption of large amounts of nonprotein nitrogen from the 0.8*N* acid extract by the protein residue was investigated. The aqueous extract from 2½ g. of meal was centrifuged, and the proteins were precipitated from the solution at pH of 4.1. The protein was carefully washed and purified by reprecipitating several times. The solution of this protein in 0.004*N* NaOH was assumed to be relatively free from nonprotein nitrogen. Water containing glycine (7.2 mg. N), glutamic acid (8.8 mg. N), and arginine monohydrochloride (10.7 mg. N) was added to the protein solution, and the mixture was shaken thoroughly. The glutamic acid lowered the pH to 4.25 and caused coagulation of the protein, which was filtered off, and a Kjeldahl nitrogen determination was made on the filtrate. It was found to contain 26.2 mg. of nitrogen. Another protein sample was treated in the same manner except that acetic acid was used to bring the pH to 4.25. This filtrate contained only 0.4 mg. of nitrogen. Thus, all but 3.4% of the added amino nitrogen was recovered in the filtrate.

Electrodialysis of Meal Extracts

For the purpose of nonprotein nitrogen determinations, differences in molecular size are of more significance than chemical differences. Some physical method of separation according to molecular size would be the best means of classifying the extracted nitrogenous compounds. Electrodialysis was the method chosen for this purpose, and in this work nonprotein nitrogen and the nitrogen which will electrodialyze through a cellophane membrane are taken as synonymous. Nonprotein nitrogenous compounds are here defined, therefore, chiefly on the basis of particle size.

The cell used was of glass and had ground glass faces between the three sections. The capacity of the center section was 85 ml. and that of the two end sections was about 40 ml. each. The electrodes were platinum disks 38 mm. in diameter placed about 2 mm. from the membranes. Since the anode and cathode wash liquids were to be analyzed for small amounts of nitrogen, it was necessary to keep the volumes of these liquids as small as possible. This was done by means of a still and a recirculating device which permitted the use of as little as 250

to 300 ml. of water in each chamber during a dialysis which lasted as long as five days. A cross-section drawing through the anode compartment is given in Figure 2 and a detailed section of the dialysis cell in Figure 3.

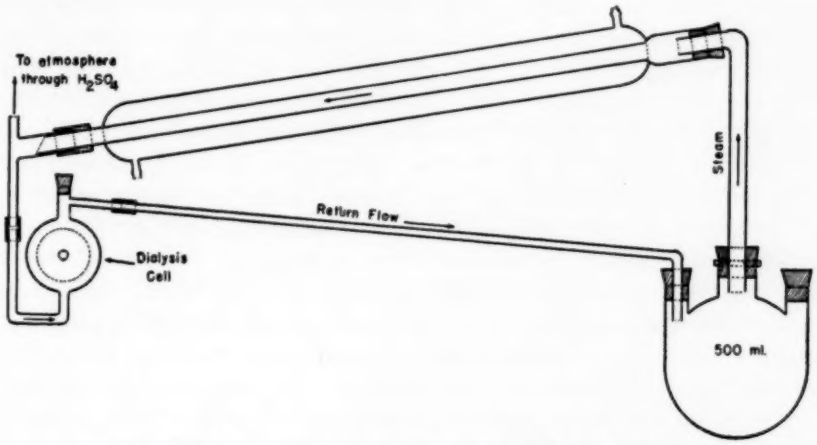

Fig. 2. Section diagram through anode compartment showing recirculating device.

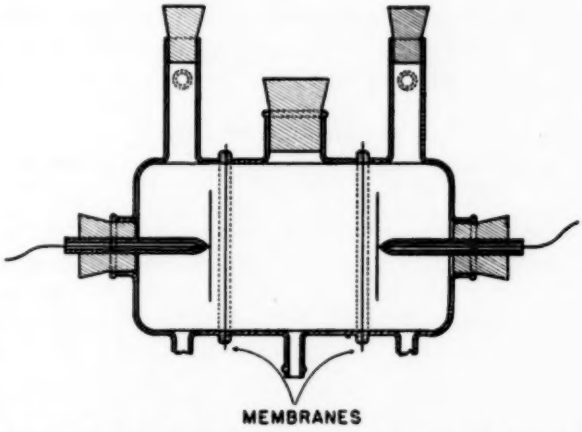

Fig. 3. Section through dialysis cell showing membranes and electrodes.

The membranes used in this work were of cellophane made especially for dialytic purposes by E. I. du Pont de Nemours and Co. A number of experiments were carried out to ascertain the approximate limit to the size of the molecules which would dialyze through this membrane. These experiments indicated that the compounds which dialyzed through were not limited to amino acids and simple peptides, but that higher-molecular-weight nonprotein compounds such as peptones could also penetrate the cellophane.

In the dialysis of the trichloracetic acid extracts, the solutions used were the clear, supernatant liquids obtained by centrifuging the meal suspensions. Enough resistance was included in the circuit so that the current flowing through the apparatus was never in excess of 0.2 ampere. The dialysis was considered to be finished when only 5 to 7 milliamperes flowed under a potential of 220 volts, since with doubly distilled water in the cell the current was 3.5 milliamperes. The time required for dialysis was, in general, directly proportional to the amount of trichloracetic acid added and ranged from 18 hours to 5 days.

At the conclusion of the dialysis, the solution in the center cell was removed, any precipitated protein filtered off, and the filtrate made to a volume of 100 ml. Aliquots of this solution and of the anode and cathode wash liquors, which had been concentrated to 100 ml., were used for duplicate micro-Kjeldahl nitrogen determinations. The rest of the solution from the center cell was treated with 20% phosphotungstic acid, and a nitrogen determination was made on the supernatant liquor obtained by centrifuging. The nitrogen precipitated by the phosphotungstic acid was assumed to be present principally in dispersed proteins and large protein fragments, while the nitrogen remaining in solution was nonprotein nitrogen that would have passed through the membrane if the dialysis could have been continued long enough. Basic nitrogenous nonprotein compounds were, of course, precipitated along with the protein.

TABLE II

DISTRIBUTION OF NITROGEN[1] AFTER ELECTRODIALYSIS OF VARIOUS TRICHLORACETIC ACID EXTRACTS OF A SAMPLE OF DUNFIELD SOYBEANS

	EXTRACTS								
	pH 0.3 (0.8*N*)	pH 4.10 (0.015*N*)	pH 1.25 (0.080*N*)	pH 1.60 (0.058*N*)	pH 4.92 (0.009*N*)	pH 5.55 (0.005*N*)	pH 5.85 (0.002*N*)	pH 5.90 (0.002*N*)	Suspension of meal in water (pH 6.50)
Anode solution	0.75	0.70	0.64	0.97	1.00	1.11	1.60	1.54	0.80
Cathode solution	2.27	2.16	2.48	2.26	2.39	2.34	2.46	2.72	2.01
Center solution	0.17	1.90	1.27	1.06	2.46	4.04	3.42	3.52	0.72
Precipitate in center	—	1.22	5.00	30.52	3.91	29.78	29.15	35.38	—
Total recovered	3.19	5.98	9.39	34.81	9.76	37.27	36.63	43.16	—
Total extracted	3.19	6.01	9.46	34.50	9.52	36.80	37.30	44.00	—
DISTRIBUTION OF DIALYZABLE OR NONPROTEIN NITROGEN									
Anode	0.75	0.70	0.64	0.97	1.00	1.11	1.60	1.54	0.80
Cathode	2.27	2.16	2.48	2.26	2.39	2.34	2.46	2.72	2.01
Center soln. after phosphotungstic acid precipitation	0.07	0.32	0.22	0.26	0.36	0.88	0.58	0.55	0.39
Total	3.09	3.18	3.34	3.49	3.75	4.33	4.64	4.81	3.20

[1] Milligrams of nitrogen per gram of meal. Total nitrogen present was 77.8 mg.

Meal extracts obtained at eight different acid concentrations were dialyzed in this manner and the distribution of nitrogen in the three chambers determined. These results are presented in Table II. The last column of figures was obtained by dialyzing a suspension of the meal in water without any preliminary separation by centrifuging.

Effect of Particle Size and Oil Content

Before an extraction procedure of this type could be of any value as an analytical method, the effect of the oil content and particle size of the meal had to be investigated. Some of the original fat-free, flaked meal was ground to different sizes in the Wiley mill, and the results obtained by extracting with 0.8*N* trichloracetic acid are shown in Table III.

TABLE III

EFFECT OF MEAL SIZE ON 0.8*N* TRICHLORACETIC ACID EXTRACTION

Meal grind	Milligrams of N extracted per gram of meal
Flakes	3.03
2-mm. screen of Wiley mill	3.00
1-mm. screen of Wiley mill	3.09
½-mm. screen of Wiley mill	3.14
100 mesh meal (ball mill)	3.19

Total nitrogen present was 77.8 mg.

The amount of nitrogen extracted from the 1-mm. Wiley screen meal is about 3% less than that extracted from the 100-mesh meal, but since all of the routine samples of the U. S. Regional Soybean Industrial Products Laboratory are ground to the former size, it was used in all subsequent work.

To determine the effect of the oil, three samples of 1-mm. Wiley screen meal of different oil content were extracted with 0.8*N* trichloracetic acid. The results, given in Table IV, show that the oil has no effect at all except to change the basis for calculating results.

TABLE IV

EFFECT OF OIL CONTENT ON TRICHLORACETIC ACID EXTRACTION

	Oil	0.8 N extract	Oil-free basis
	%	*mg. N extd./g. meal*	*mg. N extd./g. meal*
Whole meal	21.5	2.50	3.19
Percolator extracted	3.0	3.05	3.15
Soxhlet extracted	0.0	3.20	3.20

Total nitrogen present was 77.8 mg.

Since trichloracetic acid is a rather expensive reagent, it was thought advisable to determine whether smaller quantities could be used. The results were found to be the same and equally reproducible when 1 g. of meal was extracted with 40 ml. of 0.8*N* trichloracetic acid and a 25-ml. aliquot of this used for the nitrogen determination. The use of the 25-ml. portion is also advantageous in that there is much less trouble with foaming during the Kjeldahl digestion.

Trichloracetic Acid Extraction of Different Soybean Meals

A number of samples of different beans were extracted by the above-mentioned procedure to determine whether any difference in nonprotein nitrogen could be observed between the different varieties and localities of growing. A dozen samples were selected to represent, so far as possible, a high- and a low-nitrogen bean of each of several varieties. A sample of Dunfield beans was sprouted in the dark, dried at 105°C., ground in the Wiley mill, defatted by a percolator extraction, and then extracted with 0.8*N* trichloracetic acid. A control sample was treated in the same manner, except that it was not sprouted. These results and a short description of each bean are given in Table V, which also includes the nitrogen, oil, and moisture figures on the whole bean.

TABLE V

EXTRACTION OF DIFFERENT BEAN SAMPLES WITH 0.8*N* TRICHLORACETIC ACID

Samples	Fat-free meal			Whole meal		
	Total nitrogen in a gram of meal	0.8*N* extract		Nitrogen	Oil	Moisture
		mg. N/g. meal	Percent of total nitrogen extracted			
	mg.			%	%	%
No. 267—Mukden 1936—Ames, Iowa	90.3	7.04	7.80	7.68	16.48	6.45
No. 499—Mukden 1937—Lafayette, Ind.	84.9	3.10	3.65	7.07	17.30	5.75
No. 507—Dunfield 1937—Columbus, Ohio	73.2	2.11	2.88	6.00	19.50	5.48
No. 510—Mukden 1937—Columbus, Ohio	79.8	2.30	2.88	6.77	18.08	5.78
No. 517—Mukden 1937—Ames, Iowa	83.1	3.92	4.72	7.00	18.39	5.83
No. 520—Dunfield 1937—Ames, Iowa	76.0	2.78	3.66	6.13	19.92	5.74
No. 632—Illini 1937—Osceola, Ark.	66.2	3.07	4.64	5.03	23.79	5.53
No. 655—86518—1937—Ames, Iowa	85.7	6.38	7.44	7.50	15.15	6.10
No. 789—Dunfield 1937—Ames, Iowa	70.3	2.61	3.71	5.72	21.24	5.85
No. 932—Mukden 1937—Ames, Iowa	78.0	2.74	3.51	6.54	18.60	5.77
No. 935—Dunfield 1937—Sand Expt. Field, Ind.	79.3	5.02	6.33	6.89	14.47	6.00
No. 937—Dunfield 1937—Lafayette, Ind.	73.0	2.50	3.42	6.07	20.64	5.67
Ungerminated—Dunfield 1937	77.8	2.81	3.61	—	—	—
Germinated—Dunfield 1937	77.8	5.63	7.24	—	—	—

The lack of correlation between the amount of total nitrogen present in the bean and the amount removed by 0.8*N* trichloracetic acid indicates that a definite type of nitrogenous material is extracted. As a further check, the two samples highest in nonprotein nitrogen, the two

lowest, and one medium sample were extracted at a pH of about 4.1 and the extracts were electrodialyzed. The results, presented in Table VI, indicate a very definite relationship between the amounts of nitrogen extracted by 0.8*N* trichloracetic acid and the amounts of nitrogen which will electrodialyze through the cellophane membranes.

TABLE VI

THE RESULTS[1] OF DIALYSIS OF pH 4.1 TRICHLORACETIC ACID EXTRACTS OF VARIOUS BEAN SAMPLES

	Sample numbers						
	267	655	632	507	510	Germinated	Ungerminated
Anode compartment	1.03	1.43	1.03	0.78	0.73	1.15	0.66
Cathode compartment	5.86	4.76	2.39	1.66	1.63	3.89	2.20
Center compartment (not pptd. by phosphotungstic acid)	0.26	0.24	0.18	0.16	0.16	0.26	0.34
Total nonprotein nitrogen by electrodialysis	7.15	6.43	3.60	2.60	2.52	5.30	3.20
Total nitrogen extracted by 0.8*N* trichloracetic acid	7.04	6.38	3.07	2.11	2.30	5.63	2.81
Total nitrogen in meal	90.3	85.7	66.2	73.2	79.8	77.8	77.8

[1] Expressed in milligrams of nitrogen per gram of meal.

Discussion

The 0.8*N* trichloracetic acid extraction lies in approximately the center of the broad minimum of the curve of nitrogen extracted (Fig. 1) extending from 0.65*N* to 1.0*N* acid concentrations. The curve in this zone is practically a straight line and even rather large differences in acid concentration would cause no appreciable error in the amount of nitrogen extracted in this concentration range. The amount of nitrogen extracted in this minimum range is only half that removed at pH 4.1, and no protein precipitate was ever observed in the center cell after dialysis of one of these 0.8*N* extracts, whereas some precipitated protein was always observed when an extract made at pH 4.1 was used. It is assumed that the other extracts made with trichloracetic acid at concentrations between 0.5*N* and 1.0*N* would behave similarly upon dialysis. The almost complete recovery of the amino nitrogen added to the purified protein solution indicates that very little adsorption occurs in the extraction procedure. It is true, of course, that the conditions are not exactly parallel, but it would be very difficult to devise any experiment in which the protein is retained in its native form.

The dialysis of the 0.8*N* trichloracetic acid extract (Table II) shows that practically all of the nitrogen extracted is of such a nature that it will pass through the membrane. The trace of nitrogen which is left in the center would very probably dialyze through also if a sufficiently long time were allowed. No satisfactory explanation can be offered

at the present time for the increasing amounts of dialyzable nitrogen in the higher pH extracts. It is felt, however, that since the 0.8*N* acid extract, the pH 4.1 extract, and the meal-water suspension give results which check very closely, the other anomalous results do not invalidate the conclusion that the 0.8*N* acid extract contains no nitrogen but nonprotein nitrogen and practically all of the nonprotein nitrogen.

Since the 12 samples of beans selected for extraction (Table V) were grown at agricultural experiment stations under different conditions, it seems very reasonable to assume that the different varieties of beans should contain widely varying amounts of nonprotein nitrogen. There is no correlation between the amount of total nitrogen in the bean and that extracted. This fact also serves to indicate that some definite type of nitrogen or some nitrogen fraction is being preferentially extracted, and that it is not just a random solution of some of the protein. The dialysis of extracts of five of these samples made at a pH of about 4.1 (Table VI) shows that all of the nitrogen extracted by 0.8*N* trichloracetic acid and only that nitrogen will electrodialyze through the cellophane membranes.

Summary

In the extraction of soybean meal with trichloracetic acid, the minimum amount of nitrogen was removed with acid concentrations lying between 0.65*N* and 1.0*N* acid, 0.8*N* acid being taken as the midpoint. The amount of nitrogen extracted in this region was only about one-half that extracted at pH 4.1, which is the isoelectric point of soybean protein in the meal as determined by solubility measurements.

An electrodialysis apparatus, in which the water is recirculated after purification by distillation, is described. This arrangement has the advantage of keeping the volume of water circulated through the electrode chambers at a minimum.

Soybean meal was extracted with trichloracetic acid of eight different concentrations and the extracts electrodialyzed through cellophane membranes. All of the nitrogen extracted with 0.8*N* acid dialyzed through the membrane while all other extracts left some precipitated protein in the center compartment.

Several varieties of beans grown in different localities were extracted with 0.8*N* trichloracetic acid. The nonprotein nitrogen determinations indicated relatively large differences and bore no relationship to the total nitrogen present. Electrodialysis of extracts of five of these samples showed again that the amount of nitrogen extracted with 0.8*N* acid and the amount which electrodialyzed are of the same magnitude.

Details are given for a simple, rapid procedure which should serve as a routine method for determining nonprotein nitrogen in soybean meal. The nonprotein nitrogen is extracted with 0.8*N* trichloracetic acid and the nitrogen in the extract is determined directly.

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THE HEMICELLULOSES FROM OAT HULLS. II

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In a former paper (Anderson and Krznarich, 1935) it was intimated that a mixture of hemicelluloses was obtained from oat hulls. Furthermore, results seemed to indicate the presence of a polyose type of hemicellulose along with a polyuronide type. By using refinements in methods of preparation, purification, and fractionation, the author, in a continuation of work along these lines, has obtained data which show a definite separation of the two types of hemicelluloses.

Experimental

Preparation of hemicelluloses A, B, and C.—Five hundred grams of oat hulls were extracted repeatedly with acetone, hot alcohol, and boiling water. The hulls were then mixed with 7 times their weight of 5% sodium hydroxide solution and allowed to stand for 36 hours. The mixture was filtered and hemicellulose *A* was precipitated from the solution by slightly acidifying with hydrochloric acid. The precipitate was separated and completely washed in the centrifuge. Hemicellulose *B* was precipitated from the filtrate from *A* by addition of 1½ volumes of ethyl alcohol (80%–90%). The residual hulls were extracted a

second time with sodium hydroxide, and a second crop of both hemicellulose *A* and *B* was obtained in a similar manner. These will be referred to as *A'* and *B'*. The residual hulls were then extracted with 0.05 *N* hydrochloric acid, and pectin *A* was thus removed. An extraction with 5% ammonium hydroxide removed pectin *B* (Anderson, 1936). The hulls were then placed in a weak acid solution and chlorinated for one hour. Upon extraction with hot alcohol a supposed chlorine compound of lignin was removed. This was precipitated from the alcohol solution by addition of water. This material resembled that extracted from wood sawdust (Anderson, unpublished data). The residual hulls were extracted again with 5% sodium hydroxide solution and hemicellulose *C* was removed and precipitated as before. No pectic material was obtained after chlorination of the hulls. The total yield of hemicellulose was approximately 35% of the weight of the hulls used. This is a 7% increase over the amount previously reported (Anderson and Krznarich, 1935).

Fractionation and purification of the hemicelluloses.—Crude hemicellulose *A*, which showed a carbon dioxide content of 0.77% (Lefevre and Tollens, 1907; Dickson, Otterson, and Link, 1930), a pentosan of 92.49%, ash of 1.31%, and $[\alpha]_D^{20}$ in 5% sodium hydroxide of $-78.21°$, was purified by chlorination. This resulted in a hemicellulose *A* with a carbon dioxide content of 0.73%, a pentosan of 95.56%, and ash of 0.60%. The hemicellulose was dissolved in 5% sodium hydroxide solution, the solution made just acid, and chlorine gas passed in with shaking for 30 minutes. The mixture was centrifuged and the most insoluble fraction, hemicellulose A_1, was removed. The second fraction was obtained by adding $1\frac{1}{2}$ volumes of alcohol to the filtrate from A_1. Each hemicellulose, both from the first and second sodium hydroxide extractions, was treated in the same way. This resulted in fractions A_1, A_2, A_1', A_2', B_1', B_2'. Hemicellulose *B* was not fractionated since only a small amount of it was originally obtained. Results of analyses are given in Table I.

In each case a distinct fractionation is evident. Hemicellulose *A* is split into a fraction A_1, which is evidently almost a pure xylan. Although the carbon dioxide content is 0.56% (corresponding to uronic anhydride of 2.24%), it was found difficult to prepare any polyose material which gave a carbon dioxide value radically less by the method used. Mannans *A* and *B*, prepared from ivory nut waste, gave carbon dioxides of 0.45% and 0.53% respectively. Fractions A_1' and A_2' give analytical values corresponding to that of the above fraction and may be termed polyose materials. Fractions A_2, *B*, B_1', and B_2' are undoubtedly polyuronide materials, as evidenced by the increased uronic anyhydride content.

TABLE I

RESULTS OF PRELIMINARY PURIFICATION AND FRACTIONATION

Fraction	Carbon dioxide	Pentosan	$[\alpha]_D^{20}$ in sodium hydroxide	Ash
A	0.73%	95.56%	−78.21°	0.60%
A_1	0.56%	97.35%	*	1.67%
A_2	1.85%	80.93%	−83.03°	*
B	1.49%	66.93%	*	*
A_1'	0.54%	*	*	7.38%
A_2'	0.63%	*	*	*
B_1'	0.84%	*	*	*
B_2'	1.22%	*	*	*
C	0.72%	*	*	*

* Values not obtained.

Further treatment of hemicellulose A_1 by redissolving in sodium hydroxide solution, chlorinating, and reprecipitating gave two fractions containing 0.67% and 1.75% carbon dioxide respectively. The fraction analyzing 0.67% carbon dioxide was of the higher yield, approximately 55% of the original, the second being 10%.

By extracting fractions A_2', C, and B_1' with hot water, it was possible to separate each into a water-soluble and water-insoluble portion. Hemicellulose B_2' was entirely water soluble. The results of carbon dioxide determinations on each of the fractions: hemicellulose A_2', water soluble 0.94%, water insoluble 0.80%; hemicellulose C, water soluble 0.90%, water insoluble 0.61%; hemicellulose B_1', water soluble 1.21%, water insoluble 0.85%. In each case the water-soluble fraction shows a higher uronic anhydride content substantiating the results obtained above (Table I). From these data it is readily ascertainable that polyuronides are more soluble and more easily extracted than the polyoses.

Summary

Repeated fractionation of the hemicelluloses from oat hulls showed them to be composed of polyose and polyuronide material, the polyuronide type being more soluble and more readily extracted.

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DOUGH IMPROVEMENT STUDIES. I. OXIDATION OF GLUTATHIONE BY POTASSIUM BROMATE

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The presence of glutathione was actually discovered about 50 years ago. It was rediscovered and identified in 1921 by F. G. Hopkins. Glutathione is composed of three amino acids, cystine, glutamic acid, and glycine, which occur in wheat gliadin to the extent of 2.2%, 43.0%, and 0.0% and in wheat glutenin to the extent of 3.2%, 26.5%, and 0.9% (Ritter, 1939). This compound exists in a reduced form (henceforth referred to as GSH) with an open thiol-group (-SH) and in an oxidized form (to be referred to as GSSG) with an S-S bridge. An excellent survey of our knowledge of glutathione is to be found in a French monograph by Binet and Weller (1937).

It has been pointed out that "low-gradeness of flour" does not depend on the ether-extractable fraction of wheat, but on some other factor or factors. Among non-enzymatic substances proved to have a more or less deleterious effect on baking quality are asparagine (Bull, 1937) and, according to Sullivan *et al.* (1936, 1936a, and 1937), trimethylamine, guanidine and especially glutathione. Jørgensen (1935, 1935a) has already drawn attention to the importance of glutathione in baking.

Since the work of Grassmann in 1931 it is known that GSH activates the proteolytic enzymes of the papain type, which is the type found in wheat, according to the investigations of Balls and Hale (1935, 1936, 1938). Ford and Maiden (1938) interpret their results as showing that it is not activation of the papain that happens through the presence of GSH in a dough, but that most likely there is a direct action on the gluten proteins. Balls and Hale (1936a) also mention that possibly GSH activates the gluten proteins. In this connection it is interesting to note the suggestion of Albers (1936) that the activation of the papain by GSH takes place on the apo-enzyme, which would be the protein component of the enzyme. Glutathione may influence fermentation rate, but all authors do not yet agree on this issue.

An important role of glutathione is the maintenance of the reducing activity of the cell, which seems to be necessary since synthesis in the cell is a reduction process (Bertho, 1935), whereas oxidation leads to the breaking down of cell compounds. Van Laer (1935) mentions that after death the rH increases. GSH has in fact an exceptionally strong reducing power. At pH 7.0 and 37° for a 0.01 molar solution an rH as low as 9.3 has been found, whereas measurements on wheat-flour

doughs gave an rH of 15 to 18 (Van Laer, 1935a; Potel and Chaminade, 1935). For a convenient explanation of the term rH the reader is referred to Kent-Jones (1939).

Other reducing substances in a wheat-flour dough are some of the sugars present of which levulose (a component of sucrose) has specially strong reduction properties. Schoen (1938) states that the rH of glucose (dextrose) and levulose are 11.3 and less than 2.3 respectively. The latter may—at some stage in the process of fermentation—exert its influence before being itself fermented.

Experimental

The glutathione (GSH) was obtained from Hoffmann, La Roche, in Basle (Switzerland). The potassium bromate ($KBrO_3$), the maleic acid, and the copper and iron salts were normal Merck products. The buffer solutions were prepared according to Sørensen by mixing varying amounts of a 1/15 molar solution of primary potassium phosphate (KH_2PO_4) with varying amounts of a 1/15 molar solution of secondary sodium phosphate ($Na_2HPO_4 \cdot 2H_2O$).

A micro iodine titration, using starch as indicator, was used to follow the oxidation of glutathione. The reaction is $2GSH + 2I = GSSG + 2HI$. The molecular weight of GSH is 307, so that:

1.000 cc. n/500 iodine corresponds to 0.614 mg. GSH
or: 1.628 cc. n/500 iodine corresponds to 1.000 mg. GSH

Treadwell (1927) draws attention to the fact that concentration is of importance in titrations with weak iodine solutions; he reports that the blue coloration appeared after adding 0.15 cc. iodine solution when 50 cc. water was used, but only after adding 0.64 cc. when 200 cc. of water was used.

Rate of Oxidation with Bromate

The oxidation was followed for several hours using different concentrations of bromate (Fig. 1). GSH in water solution was slowly autoxidized. After 21 hours only roughly 60% of the glutathione was still present in the reduced form.

Not even with 50 times more bromate than GSH (curve 6) was the oxidation instantaneous. The fact that curve 7 (Fig. 1) never quite touched the abscissa is no doubt due to the situation observed by Treadwell (1927) as already mentioned. It is interesting to note that all along the oxidation with the highest concentration of bromate (curve 7) required only 0.05 cc. of n/500 iodine, whereas in all other cases (Figs. 1 and 2; Table II) a minimum of 0.15 cc. was required. Pure water alone needed 0.22 cc. iodine solution.

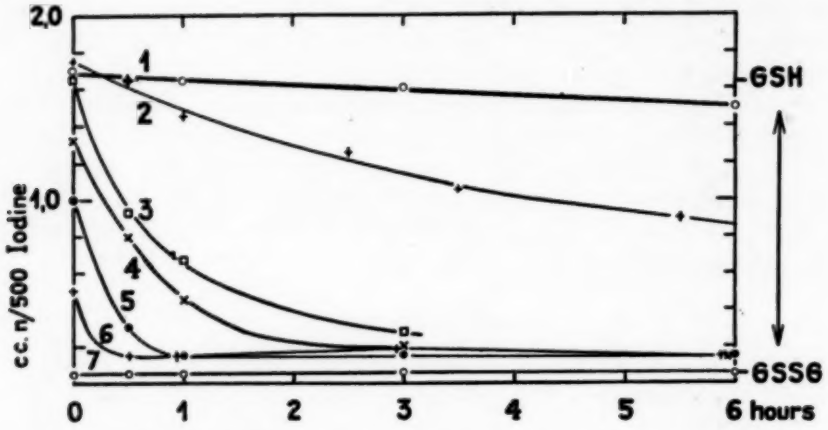

Fig. 1. Rate of oxidation of glutathione (GSH) by different amounts of bromate at room temperature. In all cases 1 mg. GSH in 10 cc. distilled water was used. The ratios of GSH to $KBrO_3$ were:

Curve 1,	1:0	Curve 3,	1:5	Curve 5,	1:20	Curve 7,	1:500
Curve 2,	1:0.5	Curve 4,	1:10	Curve 6,	1:50		

After 21 hours the cc. of n/500 iodine required for the conditions represented by the respective curves were as follows: curve 1, 1.00; curves 4, 5, and 6, 0.10; curve 7, 0.05, and curve 2 (after 8 hours), 0.7 cc.

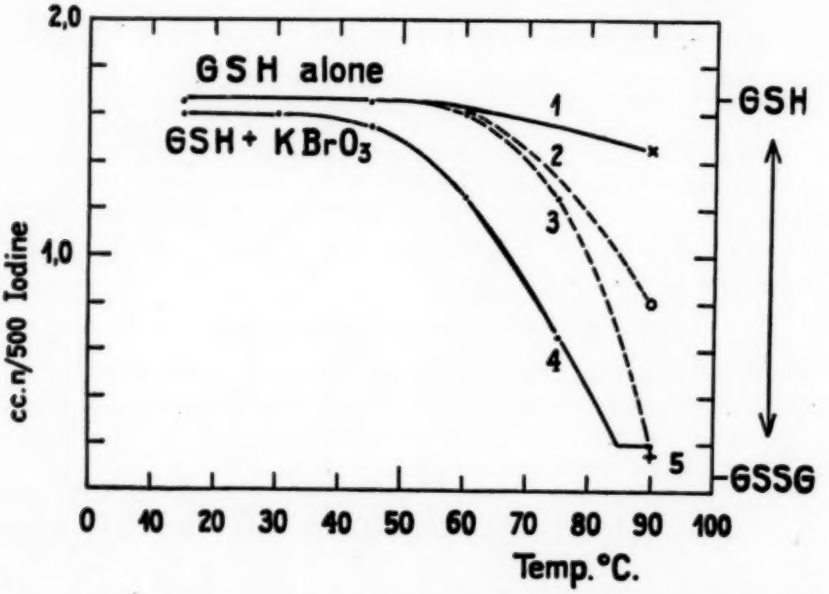

Fig. 2. Influence of temperature on the oxidation of 1 mg. glutathione (GSH) by 0.5 mg. bromate in 10 cc. distilled water. Reaction time 10 minutes. Cooled before titration.

Curve 1, GSH alone in CO_2 saturated distilled water.
Curve 2, GSH alone in boiled distilled water.
Curve 3, GSH alone in distilled water, not previously boiled.
Curve 4, GSH + $KBrO_3$ in distilled water, not previously boiled.
Point 5, GSH + $KBrO_3$ in CO_2 saturated distilled water.

Influence of Hydrogen-Ion Concentration

According to Harington and Mead (1935) an aqueous solution of GSH has a pH of about 2.5. Read and Haas (1938) state that free bromic acid is formed from bromate only at a very low pH.

TABLE I

INFLUENCE OF pH ON THE OXIDATION OF GLUTATHIONE (GSH) SOLUTION ALONE

(Buffer solutions consisted of Sørensen's phosphate mixtures. Reaction time 5 minutes.)

pH	10 cc. buffer solution + 0.5 cc. $KBrO_3$	10 cc. buffer solution + 1 mg. GSH	10 cc. buffer solution + 1 mg. GSH + 0.5 mg. $KBrO_3$
	cc. n/500 iodine		
8.0	0.15	(3.30)[1]	(3.50)
7.0	0.10	(2.55)	(2.68)
6.2	0.10	(2.05)	(2.07)
5.6	0.10	1.75	1.75
5.0	0.10	1.75	1.60
4.5	0.10	1.75	1.65

[1] Parentheses denote that the exact end point of the titration was difficult to fix, because the blue coloration disappeared slowly again.

TABLE II

OXIDATION OF GLUTATHIONE (GSH) BY BROMATE IN THE PRESENCE OF CERTAIN SUBSTANCES AT ROOM TEMPERATURE

(In all cases 1 mg. GSH in 10 cc. distilled water)

No.	$KBrO_3$	Addition	Reaction time in minutes 0	5	60
	mg.		*cc. n/500 iodine*		
1	5	None	1.70	1.60	0.70
2	5	9 mg. fresh baker's yeast	1.65	1.65	1.10
3	5	6 mg. NaCl	1.65	1.60	0.95
4	5	15 mg. NaCl	1.65	1.60	0.95
5	5	2.5 mg. copper chloride [1]	1.85	1.65	0.60
6	5	8.4 mg. ferric sulphate	1.25	0.20	0.20
7	5	4.0 mg. ferrous sulphate	1.20	0.15	0.15
8	5	1.0 mg. ferrous sulphate	1.35	0.25	0.25
9	0.5	None	1.65	1.65	1.45
10	0.5	2.5 mg. copper chloride [1]	1.80	1.60	1.25
11	0.5	8.4 mg. ferric sulphate	1.25	0.95	0.15
12	0.5	4.0 mg. ferrous sulphate	1.55	1.15	0.15
13	None	2.5 mg. copper chloride [1]	1.85	1.60	1.50
14	None	8.4 mg. ferric sulphate	1.40	1.00	0.60
15	None	4.0 mg. ferrous sulphate	1.60	1.60	1.60
16	None	None	1.70	1.65	1.65

[1] In presence of copper chloride (Nos. 5, 10, and 13) the blue coloration keeps on disappearing, making it hard to fix the end point of the titration.

Unfortunately no apparatus was at hand for the precise control of pH. The figures in Table I show that oxidation is very slow between

pH 4.5 and 5.6. Above about pH 6 probably a rapid but partial decomposition of the GSH took place. The optimum for the oxidation to GSSG by oxygen is given as pH 7.4 by Dixon and Tunnicliffe (1923). At higher pH there is said to be a cleavage of the molecule with the formation of sulphenic, sulphinic, and sulphonic acids.

Influence of Temperature

It has already been mentioned that GSH alone in water autoxidized to the extent of 60% in 21 hours at room temperature (Fig. 1). The autoxidation is rapid above 60° (Fig. 2). Ten minutes at 90° is sufficient to completely oxidize GSH if water containing a certain amount of absorbed oxygen is used. If the latter is driven out by boiling previous to the addition of GSH a partial oxidation takes place, probably due on the one hand to the incomplete expulsion of all traces of oxygen, and on the other hand to the contact of the liquid surface with the atmosphere, in the presence of traces of both copper and iron compounds. Treatment at 90°C. for 10 minutes in an atmosphere of carbon dioxide considerably reduced the degree of oxidation.

In the presence of a small amount of bromate, oxidation of GSH was noticeably accelerated above 40°C. Nevertheless it needed a temperature of 85°C. to completely oxidize the GSH in that short a time. These tests again show the slowness of the oxidation of GSH by bromate as compared with oxidation by iodine, even if heat is used.

Influence of Some Catalysts

Results given in Table II show that, with the amounts used and with reaction times up to one hour, neither baker's yeast nor sodium chloride had any accelerating influence on the oxidation of GSH with bromate. As was expected, yeast, which contains GSH itself, increased the amount of iodine required. It is known that copper and iron play an important part in biological oxidations. As a catalyst of the bromate oxidation copper chloride seemed to have no effect in our tests. On the other hand both Fe^{++} and Fe^{+++} salts acted as strong catalysts. The copper and ferrous (Fe^{++}) salts alone had no action on GSH, whereas the ferric (Fe^{+++}) salt alone oxidized roughly to the same extent as bromate did. Binet and Weller (1936) used ferric chloride to completely oxidize GSH in 24 hours.

Next it was thought that some compound in flour might activate the oxidation of a bromate-treated dough. The water-extractable part of a short-patent flour did not seem to exert any significant influence on the oxidation rate, as figures in Table III show.

TABLE III

INFLUENCE OF FLOUR EXTRACT ON THE OXIDATION OF 1 MG. GLUTATHIONE (GSH) BY 0.5 MG. BROMATE IN 10 CC. DISTILLED WATER

(10 g. short-patent flour extracted by 50 cc. distilled water at 27°C. for 1 hour; filtration 20 minutes; in all cases 5 cc. flour extract used)

			cc. n/500 iodine		
GSH	$KBrO_3$	Reaction time	Actual titration	Cc. used by flour extract deducted	Without flour extract (from Fig. 1)
mg.	*mg.*	*hrs.*			
–	—	0	0.60	—	—
1	—	0	2.00	1.40	1.65
1	0.5	0	1.90	1.30	1.75
1	0.5	1	1.95	1.35	1.50
1	0.5	2	1.90	1.30	1.30
1	0.5	3	1.70	1.10	1.15

Influence of Different Oxidizing Agents and Maleic Acid

Bromate and iodate, well-known improvers in the baking industry, also completely oxidized glutathione (Table IV). Chlorate, on the other hand, is known to have no improving action in a dough and did not appear to oxidize the glutathione in the least.

TABLE IV

INFLUENCE OF A FEW OXIDIZING AGENTS AND MALEIC ACID ON THE OXIDATION OF GLUTATHIONE (GSH)

(50 mg. of each substance dissolved in 10 cc.)

Product added	GSH	Reaction time	n/500 iodine	Degree of oxidation
	mg.	*hrs.*	*cc.*	
None	1	1	1.65	——
Potassium chlorate	1	1	1.60	No oxidation
Potassium bromate	1	1	0.15	Complete
Potassium iodate	1	1	0.15	Complete
Dehydroascorbic acid	1	1	0.80	50%
Maleic acid	–	0	0.15	No effect on iodine
Maleic acid	1	1	1.90	No oxidation

Melville and Shattock (1938) found that the oxidized form of vitamin C, dehydroascorbic acid, is just as efficient an improver as bromate. For our titration 50 mg. of ascorbic acid was first completely oxidized with iodine before being brought together with 1 mg. of glutathione. In spite of this larger amount of dehydroascorbic acid, as compared with the amount of bromate, the rate of oxidation was only half that of the bromate (Table IV).

TABLE V

BAKING TESTS WITH BROMATE AND MALEIC ACID AS IMPROVER

(100 g. flour, 2 g. salt, 3 g. yeast, 0.5 g. malt flour, 65 cc. water; 2 hours fermentation; proof as needed)

	Wheat flour	Untreated	Bromate 2 mg.	Maleic acid 2 mg.	Maleic acid 5 mg.	Maleic acid 10 mg.
Loaf vol., *cc.*	Canadian	680	720	650	700	—
Loaf vol., *cc.*	Argentine	410	460	435	430	430
Oven spring		Fair	Excellent	Unsatisfactory		
Texture		Fine	Very fine, silky	Coarse		

Recently Morgan and Friedmann (1938, 1938a) showed that on addition of maleic acid to thiol compounds (to which class glutathione also belongs) α-thiosuccinic acid derivatives are formed. Such an addition product with glutathione was isolated. Furthermore the authors found that enzymic actions induced by SH-compounds were inhibited by the addition of maleic acid ($HOOC \cdot CH = CH \cdot COOH$). If that is the case, it might be possible to so bind the glutathione in dough that it can no longer activate the proteolytic enzymes of wheat flour, which, according to Jørgensen's (1935) theory, would bring about and improvement in baking quality. Table IV shows that on the addition of the acid the reducing power of glutathione was not diminished at all. Baking tests (Table V) also brought out the difference between a treatment with bromate and with maleic acid. On the addition of the latter none of the characteristics of improved doughs were observed. In a wheat-flour dough maleic acid would not seem to react according to the manner indicated by Morgan and Friedmann (1938a).

Summary

For over 20 years potassium bromate has been widely used to improve the baking quality of wheat flour. An explanation of the reaction has only recently been given by Jørgensen (1935).

It was thought of interest to study the rate of oxidation by bromate of pure reduced glutathione, since the latter is probably the main factor responsible for the "low-gradeness of flour" (Sullivan, Howe, and Schmalz, 1936a).

Glutathione in water solution was autoxidized to the extent of 60% in 21 hours at room temperature. In a water solution saturated with carbonic acid autoxidation was slow, even at 90°C.

At temperatures below 40°C. an enormous overdose of bromate is necessary for the rapid oxidation of glutathione, as compared with iodine, which oxidizes immediately. A treatment of 10 minutes above 40°C. greatly accelerated the oxidation, which was completed in that lapse of time at 85°C.

Below a pH of 5.6 the hydrogen-ion concentration had no marked influence on the oxidation with bromate. Above pH 6.2 glutathione itself was probably broken down.

Whereas copper chloride seemed to have no effect on the rate of oxidation, ferric and ferrous sulphate both exerted a significant accelerating action on the oxidation. The water extract of flour did not influence the rate of oxidation.

Maleic acid is said to form an addition product with glutathione, inhibiting the activating properties of the latter on enzyme action. It was found that maleic acid had no influence on the reducing power of pure glutathione, nor did it communicate any of the characteristics of bromate treatment when added to a wheat-flour dough.

Bromate is known to work gradually as an improver in wheat-flour dough. This can be explained by its slow rate of oxidation of glutathione.

Acknowledgment

The author wishes to acknowledge the help and suggestions of Mr. F. Brassel in carrying out the work reported in this paper.

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POLARISCOPIC DETERMINATION OF PROTEOLYTIC ACTIVITY

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(Read at the Annual Meeting, May 1939)

Both physical and chemical methods have been used extensively in determining proteolytic activity. Generally speaking, the physical methods are more sensitive than chemical methods. The rupture of a relatively small number of peptide bonds, for example, may change the physical property of the protein to a marked extent. It is well known that gelatin is a protein which is particularly susceptible to such changes. Concurrently with the setting of gelatin other properties show equally wide changes. The optical rotatory power of gelatin is one which is no exception.

Smith (1919) determined the polarization of gelatin as a function of temperature and found that there was a very large change between 10° and 25°.

Gore (1929) showed that this large change was destroyed when the

gelatin was digested with proteolytic enzymes, and furthermore that there was a regular relationship between the activity of the enzyme and the change in polarization.

Principles of the Method

The marked effect on the polarization of gelatin as shown in Figure 1 can be utilized as a method of determining proteolytic activity. Experimentally it has been found that about 2% is the greatest concentration that can conveniently be used, because the solutions may

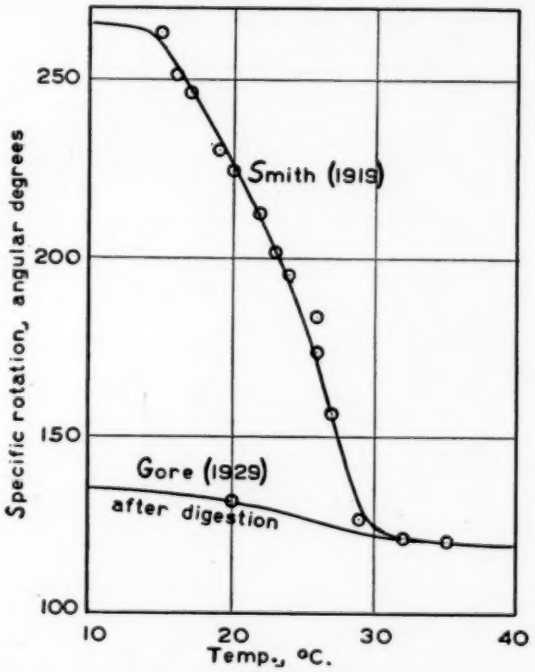

Fig. 1. Polarization of gelatin.

become too viscous to handle with greater concentrations. Probably the best method of stopping the reaction is by chilling. If the flask containing the reaction mixture is vigorously rotated in ice water, not more than one to two minutes is required to reduce the reaction velocity to one-tenth of its value at, say, 40°. The reaction may also be stopped by heating. Experimentally it has been found that the conversion occurring during the time required to raise the temperature from 40° to 80° by placing the flask in a boiling water bath cannot be detected in comparison with that occurring during an extended digestion period. Some malts must be treated in this matter in order to obtain a clear filtrate. Gore (1929) originally used 45° for the digestion, but it was

found that over long periods of time a slight destruction of enzyme occurred.

In order to be able to measure low activities a relatively long period of digestion must be chosen. Five hours has been tentatively considered as a convenient time. After digestion it would be preferable to read the polarization at, say, 15° or lower. Unless, however, one has a suitably conditioned room, fogging of the end glasses makes it almost impossible to read successfully at that temperature. Consequently 20° has been chosen as a convenient polarization temperature. As shown in Figure 1 the specific rotation changes over a 40% range.

Standardization

It has been shown that one of the most satisfactory methods of standardizing an empirical procedure such as this is to relate the activity to the initial rates of conversion (Johnston and Jozsa, 1935).

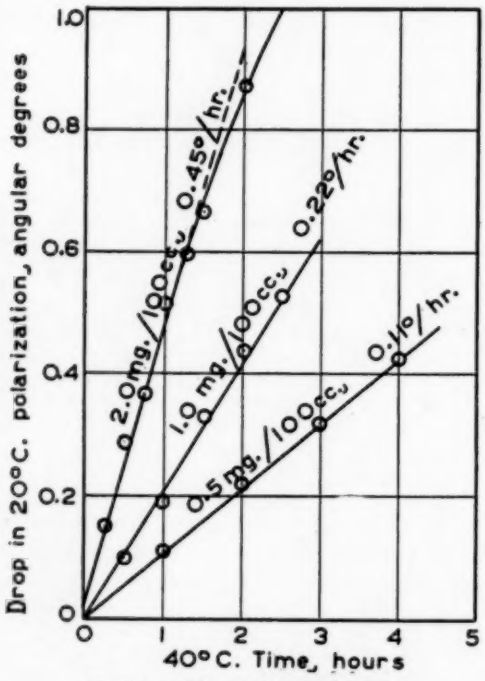

Fig. 2. Initial rates (standardization).

In this particular case it was desired to relate the activities observed to a scale which had previously been in use. A sample of known activity on this scale, namely, 25,000 milliunits per gram, was run at various concentrations. The curves in Figure 2 show respectively the

rates of conversion when the infusion contained 50, 25, and 12.5 milliunits of enzyme per 100 cc. of solution. The data lead to an average value of 0.0089° polarization change per hour per milliunit per 100 cc.

Calibration and Procedure

The adopted details of the method may be a compromise between a number of factors which need not be listed for present purposes. A sample of a commercial food gelatin containing 10% moisture, 1% ash, and having a pH of 4.5 is used as substrate. This is made up to 2 g. of gelatin in 100 g. of solution, and buffered to a pH of 4.8 with acetate buffer. It is advisable to soak the gelatin in cold water before warming to 40°. Fresh substrate should be prepared for each day's run and toluene is used to prevent growth of organisms. To a 25-cc. portion of this substrate is added 25 cc. of enzyme infusion prepared in 2.5% sodium chloride solution, both at 40°, using 50-cc. stoppered Erlen-

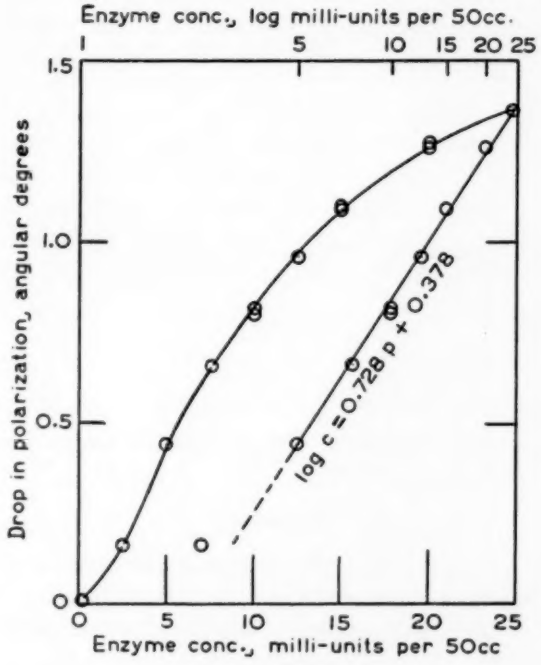

Fig. 3. Calibration.

meyers. After digestion for five hours the flasks are cooled rapidly in ice water and allowed to refrigerate for at least one hour. Alternatively, when excessively turbid enzyme extracts are used the reaction flask is placed in a vigorously boiling water bath for a few minutes until the "break" occurs, *i.e.*, until the soluble protein coagulates and floc-

culates. The flask is then cooled and allowed to refrigerate for one hour. It is then placed in a 20° bath and polarized when equilibrium is attained. Usually this requires less than one hour. Filtration of turbid conversions can conveniently be made at 20°, but time must be allowed to attain equilibrium after filtration in case the solution has warmed up during the process. Both non-gelatin and non-enzymic blanks are run concurrently. The non-enzymic blank must be poured into a polariscope tube within one minute after cooling or it will gel. Using the sample from which the curves in Figure 2 were made, the data represented in Figure 3 were obtained. Over a range from approximately 10 to 25 milliunits per 50 cc. of reaction mixture the activity can be calculated by using the formula. At lower activities this function is invalid, but an estimate could be obtained by reading the data directly from the curve. A calibration performed three months after the one shown in Figure 3 with the procedure slightly modified for excessive summer temperatures gave $\log c = 0.693p + 0.384$, which is satisfactory agreement for this type of procedure. Some typical results are given in Table I. The procedure lends itself

TABLE I

Material	Concentration	Activity
	gm. per 25 cc. infusion	*M.U./gm.*
Papain No. 1	0.00075	24000
Papain No. 1 activated with H_2S	0.00020	104000
Papain No. 2	0.00050	58000
Brewer's malt No. 1	2.0	4.1
Brewer's malt No. 2	2.0	3.6
Distiller's malt	2.0	6.1
Experimental malt No. 1	2.0	7.7
Experimental malt No. 2	2.5	1.3

readily to studies of activation. Normal flours are too low in activity to be tested by this method.

Summary

The specific rotation of gelatin at temperatures below 25° is reduced by enzymic digestion. An empirical procedure relating the extent of the decrease to the concentration of enzyme is described.

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THE NUTRITIVE VALUE OF THE PROTEINS OF RICE AND ITS BY-PRODUCTS.[1] II. EFFECT OF AMINO ACID ADDITIONS ON GROWTH

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In a previous communication (Kik, 1939) the results were reported of a study of the biological value of the proteins of rice and its by-products, and it was found that at a 5% protein level, the proteins of whole rice and those of polished rice had a lower biological value and a higher digestibility than those of rice bran and of rice polishings.

Digestibility and amino acid content of the food proteins are limitations of their nutritive value, and since both whole-rice and polished-rice proteins showed a high digestibility, it was believed necessary to study the effect of amino acid additions.

A study of the literature revealed that amino acid deficiencies have been found in garden peas (both fresh and canned), potatoes, beef, wheat, corn, and oats (Beadles, Braman, and Mitchell, 1930; Mitchell and Smuts, 1932), and hegari (Smith and Roehm, 1937).

This paper deals with a study of the effect on growth in rats fed rice diets with and without certain amino acid additions. The composition of the rations used in these experiments is given in Table I.

TABLE I

COMPOSITION OF THE RATIONS

Ration	N	Salt mixture	Butter-fat	Starch
	%	%	%	%
Whole rice (88.0%)	1.05	4.0	8.0	—
Polished rice (88.0%)	0.92	4.0	8.0	—
Rice bran (67.0%)	1.32	4.0	8.0	21.0
Rice polishings (65.0%)	1.32	4.0	8.0	23.0

The amino acids, cystine, tyrosine, tryptophane, and the "leucine fraction" (a mixture of leucine, isoleucine, and valine) were prepared in the laboratory according to standard methods (Cole, 1933) and methionine and lysine were obtained from a reliable source.[2]

[1] Research paper No. 668, Journal Series, University of Arkansas.

[2] Amino Acid Manufacturers, Chemistry Department, University of California, Los Angeles, California.

TABLE II

AVERAGE EFFECT ON GROWTH OF THE ADDITION OF CYSTINE, METHIONINE, LYSINE, GELATIN, TRYPTOPHANE, AND "LEUCINE FRACTION" TO THE PROTEINS OF WHOLE RICE (6.0%), POLISHED RICE (5.5%), RICE BRAN (8%), AND RICE POLISHINGS (8.0%) AS SHOWN BY PAIRED-FEEDING EXPERIMENTS

Series	No. prs.	Ration [1]	Initial wt.	Final wt.	Gain	Duration of experiments	Food intake	Supplemental effect	P [2]
			g.	g.	g.	*days*	g.		
1	12	WR	61	125	5.0	84 for prs. 1–9	606	+	.00018
		WR+cystine	61	130		63 for prs. 10–12			
2	6	WR	47	146	7.0	90	883	+	.00180
		WR+methionine	47	153					
3	3	WR	42	125	None	100	704	None	—
		WR+tryptophane	42	125					
4	6	WR	53	140	4.0	44 for prs. 1–3	927	+	.00270
		WR+gelatin	53	144		57 for prs. 4–6			
5	3	WR	53	164	7.0	63	988	+	—
		WR+lysine	53	171					
6	12	PR	49	107	4.0	105 for prs. 1–9	698	+	.00271
		PR+cystine	49	111		63 for prs. 10–12			
7	6	PR	52	123	7.0	63	606	+	.00010
		PR+methionine	52	130					
8	3	PR	41	101	−2.0	63	474	None	—
		PR+tryptophane	41	99					
9	3	PR	45	93	9.0	66	487	+	.003
		PR+gelatin	45	102					
10	3	PR	52	114	11.0	63	557	+	.0034
		PR+lysine		125					
11	6	PR	51	105	1.0	112	682	None	.0581
		PR+leucine fraction		106					
12	3	RB	46	175	−1	48	644	None	—
		RB+cystine		174					
13	3	RP	47	177	None	61	659	None	—
		RP+cystine		177					

[1] WR = whole rice. PR = polished rice. RB = rice bran. RP = rice polishings.
[2] The probability P is obtained according to Student's method (1908) and calculated from the differences between the average gains obtained. It is a criterion of high significance if smaller than .03.

Plan of Experiment and Results

The paired-feeding method of Beadles, Braman, and Mitchell (1930) was employed; by this method the same amount of feed is supplied to both rats of any one group. The control animals of each litter-mate group received an additional 25 mg. of amino acid daily.

The animals were confined in individual cages and the feed and animals were weighed twice weekly. All animals received daily an ample supply of vitamin B consisting of 3 cc. of an aqueous extract of 5% brewer's yeast, 1 cc. of a riboflavin concentrate corresponding to 10 micrograms, 1 cc. of an extract of vitamin B_1 crystals (Merck) furnishing 20 micrograms, and 4 drops of cod liver oil as an additional source of vitamins A and D. Only male rats were employed of an initial weight of about 50 g.

Whole rice.—This was investigated in five series of paired-feeding experiments, in which additions were made of cystine, methionine, tryptophane, gelatin, and lysine. The results are summarized in Table II, which shows the number of pairs employed in each series, the average initial and final weights of the rats, the food intake, duration of each series, and the average differences between the gains in weight. A statistically significant supplemental effect was obtained when additions were made of cystine, methionine, gelatin, and lysine.

Polished rice.—In six series of experiments, additions were made of cystine, methionine, tryptophane, gelatin, lysine, and "leucine fraction," and statistically significant results were found for the amino acids, cystine, methionine, and lysine. Gelatin, a poor protein rich in lysine, had a supplementary effect.

Rice polishings and rice bran.—Three pairs of rats were employed in each series and cystine was added to the ration of the pair mates. No beneficial effect on growth was observed.

Summary

Paired-feeding experiments showed that cystine, methionine, and lysine supplemented the proteins of whole rice and of polished rice to a slight but statistically significant extent. Tryptophane, however, did not have any beneficial effect.

Cystine did not promote growth as a supplement to the proteins of rice bran and rice polishings.

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EVIDENCE IN SUPPORT OF A RECENT PAPER CONCERNING THE EFFECT OF MILK ON THE BROMATE REQUIREMENTS OF FLOURS

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(Received for publication February 10, 1940)

Recently Ofelt and Larmour (1940) showed that in general the addition of milk solids to a modified "basic" formula increased the amount of potassium bromate necessary to produce a loaf of maximum volume by the conventional basic procedure. Their modification of the "basic" formula of the American Association of Cereal Chemists consisted of decreasing the yeast from 3% to 2%, increasing the sugar from 2.5% to 6%, increasing the salt from 1% to 1.75%, and superimposing 3% shortening and 6% powdered skimmilk. A series of baking tests was made first with the indicated formula and second with the identical formula less the milk powder—with potassium bromate as a common variable ingredient. We quote from their summary:

> In general the inclusion of 6% dry-milk solids creates a tolerance towards bromate which tends to prevent damage to loaf volume and to grain and texture when large dosages of this reagent are used. Even when the effect is small for loaf volume it remains marked for grain and texture. This buffering effect towards bromate has important commercial significance because it provides a safeguard against the possibility of damaging flours that have already been brought close to their optimum "oxidation" condition by bleaching or the addition of other oxidizing agents.

In view of the importance of this finding it seems worth while to present certain incidental data in support of their work. As in the study of Ofelt and Larmour, our baking procedure was a modification of the standard method approved by the American Association of Cereal Chemists. One hundred grams of flour (15% moisture basis) and the following percentage ingredients based on flour weight were employed: distilled water 60% (1% additional for each 1% of powdered skimmilk), 3% yeast (type A as classified in a recent article by this writer, 1940), 2½% sucrose, 1% salt, 3% shortening (hydrogenated cottonseed) when indicated and 6% powdered skimmilk when indicated. For the purpose of this paper we will consider the above a

"commercial" formula and when neither milk nor shortening is used the formula will be considered a "basic" formula. Unfortunately the data which we are about to consider were not acquired with the present purpose in mind and hence suffer a certain degree of incompleteness. Nonetheless, as can be seen from Table I, particularly when comparing the results obtained via the "commercial" versus the "basic" formula, our results are in complete agreement with those of Ofelt and Larmour

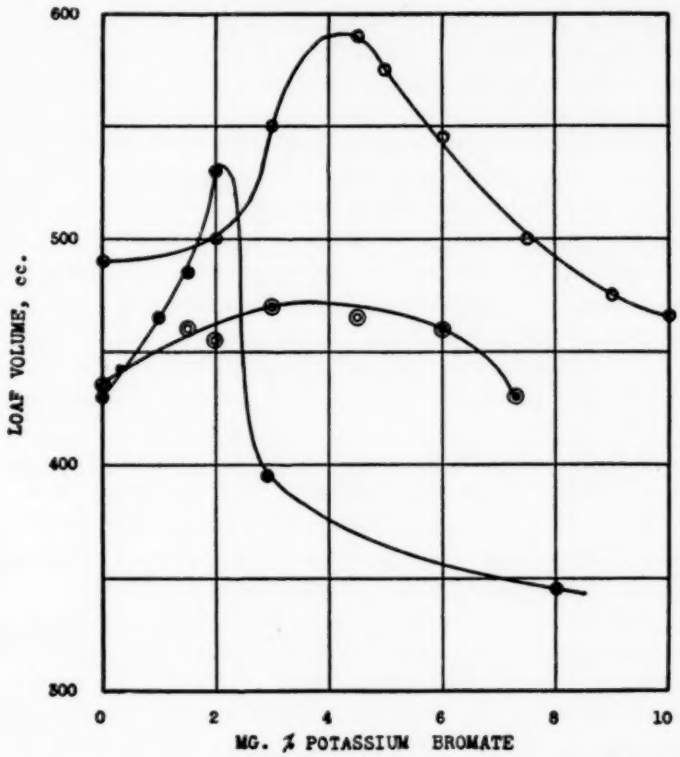

Fig. 1. Closed circles, "basic formula"; open circles, "commercial formula"; double circles, "basic formula plus 6% milk powder." The increased tolerance and response to potassium bromate in the presence of milk and shortening is evident as is the increased tolerance but decreased response to powdered skimmilk alone.

—that is, if one may assume that the "basic" and the "basic plus shortening" formulas respond similarly to increments of potassium bromate. This assumption is justified in part by the two figures for shortening plus potassium bromate in Table I and in part by the resemblance of the "basic" and the "commercial" loaf-volume versus percentage potassium bromate curves in Figure 1 to certain of the corresponding figures in the paper under consideration.

TABLE I

LOAF VOLUME AS A FUNCTION OF FORMULA AND % POTASSIUM BROMATE

Bromate, *mg.* %	0.0	1.0	1.5	2.0	3.0	4.5	5.0	6.0	7.5	8.0	9.0	10.0
Basic	430	465	485	530	395	—	—	—	—	345	—	—
6% milk powder	435	—	460	455	470	465	—	460	430	—	—	—
Shortening, 3%	445	—	—	540	—	—	—	—	—	—	—	—
Commercial	490	—	—	500	550	590	575	545	500	—	475	465

On studying Table I in its entirety, however, one finds that the increased response and tolerance to potassium bromate in the presence of milk powder and shortening cannot be attributed to the milk powder alone. The use of only milk powder increases the tolerance to potassium bromate tremendously in that 0.0075% potassium bromate produced a loaf of volume identical to that produced in the absence of this salt, whereas in the "basic" formula this quantity of potassium bromate stimulated a distinct negative response. Milk powder itself, though apparently increasing the tolerance, actually decreases the response to potassium bromate.

Within these limitations, Figure 1 constitutes a remarkable corroboration of the figure on page 7 of Ofelt and Larmour's paper insofar as the "commercial" and the "basic" formulas are concerned. Where loaf volume versus potassium bromate for the case of "milk powder" versus the "basic" formula is indicated (double circles versus closed circles, Figure 1), a somewhat different interpretation of the bromate-milk effect may obtain. The problem of potassium bromate is indeed complex and these results may prove difficult to interpret on a basis of enzyme kinetics alone. Shortening, from these meager data, appears to play a supporting role in bromate action—at least in the presence of milk solids, if not in their absence.

Several questions of interest in addition are suggested by Figure 1. For example, with the yeast and flour (one very likely milled from Inter-mountain winter wheat and of 13.0% protein and of 0.41% ash) under consideration, a volume response of 120 cc. is shown merely by the addition of 3% shortening to a formula containing 6% milk powder and 0.004% potassium bromate. Further work in this direction may possibly lead to the development of a baking test of high sensitivity to shortening characteristics. Secondly, if, as may be postulated for the moment, "basic" and "basic plus shortening" loaf volumes are similar functions of potassium bromate content, then a volume response of the order of 215 cc. may be expected by superimposing 6% milk powder upon a formula containing 3% shortening and 0.004% potassium bromate. Hence a method of unusual sensitivity to milk charac-

teristics is indicated. Thirdly, the volume response inspired by milk powder and shortening as ingredients supplementary to the "basic" formula appears to be a rather critical function of the quantity of potassium bromate present. It may in fact be negative for certain percentages of potassium bromate and be positive for either larger or smaller quantities. If, therefore, as is already accepted, the volume response to potassium bromate is at least a rough measure of the "age" or past bleaching treatment of a flour, then we may expect either negative or positive volume responses to the addition of milk powder and shortening to random flour samples. And lastly, since our selection of 6% milk powder and 3% shortening as quantities suitable for study was entirely fortuitous insofar as this subject is concerned, another promising field of work is hereby predicted—that is, the problem of determining the relative interactions of shortening and milk powder with the view of discovering whether or not a relationship exists between the optimum quantity of the one as a function of any selected quantity of the other.

These four suggestions have engaged this laboratory's attention for some time past, but unfortunately other interests have prevented and apparently will continue to prevent their study here. We therefore pass them on with the hope that other workers in a better position than we to attempt the necessary pure research will carry on. Certainly a new key to the study of the bromate effect lies here.

Summary

This writer agrees with the opinion of Ofelt and Larmour that their discovery of the "buffering" effect of milk powder to increments of potassium bromate is one of prime importance. Corroborative evidence is presented. A suggestion with supporting evidence that shortening is deeply involved in the effect is made.

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QUALITY STUDIES ON NORTH DAKOTA DURUM WHEATS (1938 CROP)

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The principal area of durum wheat production in the United States lies in the north-central section of the state of North Dakota. In this region the environmental conditions of soil and climate are conducive to the production of high-quality durum for macaroni manufacture. Despite the importance of this grain crop to North Dakota, until quite recently no satisfactory facilities for making quality studies on durum wheats in line with commercial procedures have been available. This fact was due principally to a lack of published information regarding the experimental processing of macaroni. Macaroni products of various types had been grouped together under the general designation of "alimentary pastes," under such names as macaroni, spaghetti, vermicelli, etc., to describe specific products. LeClerc (1933) proposed the adoption of the term "macaroni" or "macaroni products" as a general designation for this class of food products. When a specific physical form is referred to, it is indicated by the conventional name.

Fifield (1934) and Fifield *et al.* (1937) discussed experimental apparatus and methods for the manufacture of macaroni products and reported the results obtained upon durum wheats grown in the hard red spring wheat region of the United States from 1932 to 1936. The accumulated data presented showed that inherent quality differences exist between varieties, and that these differences are surprisingly consistent over wide ranges of seasons and environments.

Binnington and Geddes (1936) have described in detail experimental milling and processing equipment for durum wheats, based upon commercial procedures. Their article also contains a critical discussion of the methods employed in standardizing the various operations involved. Several photographs of equipment are included in their paper. The effects of variations in absorption, kneading time, and rest period and the effect of press temperature upon color characteristics, as measured by a spectrophotometer, were investigated and these variables standardized to produce optimum results in terms of color analysis. A statistical basis for establishing criteria of the differences required for distinguishing between different samples is presented. Binnington and Geddes (1937) examined 34 samples of experimentally grown durum wheats produced in Canada during 1934 and 1935, using the standardized methods previously developed. Color

analyses were made and a single-figure color estimate developed, which corresponded satisfactorily with visual color score. Quality differentiation, in terms of color and appearance, was demonstrated between the samples. Little relationship appeared to exist between the carotenoid content of durum wheat and the color of the macaroni product derived from it.

Borasio (1935) published a report dealing with the determination of the cooking quality of alimentary pastes and described methods developed for their investigation. This work was reviewed in some detail by Binnington, Johannson, and Geddes (1939) and Harris and Knowles (1939). Borasio listed the principal factors of interest from the cooking standpoint as: (1) degree of cooking required, (2) resistance to disintegration, (3) capacity for absorption of water, and (4) increase in the volume of the paste.

C. E. Mangels and E. Latzke (unpublished data, North Dakota Agricultural Experiment Station, 1934) attempted to develop a method for determining quantitatively the cooking quality of macaroni products. These workers measured the increases in length and diameter of 7-centimeter lengths of macaroni after cooking. The increase in weight during cooking was ascertained, as well as the degree of disintegration of the sample.

An additional test of cooking quality was developed by Binnington, Johannson, and Geddes (1939) for measuring the tenderness of cooked macaroni. These workers constructed an apparatus modeled after the instrument described by Bonney, Clifford, and Lepper (1931) for testing the tenderness of canned fruits and vegetables, but containing some additional features. A full description of this equipment, including a photograph and plan of construction, is included in their paper. Refined methods for determining dry volume, water absorption, increase in volume, and degree of disintegration upon cooking are also given.

Further studies of the relative macaroni-making qualities of a number of durum wheat varieties were published by Binnington and Geddes (1939). From the results of these studies, the authors emphasized the point that macaroni quality cannot as yet be predicted from a single analytical test applied to the wheat and that wheat carotene is valueless as an index of macaroni color, particularly for intervarietal prediction.

Experimental Material and Methods

Seventeen samples [1] of various varieties of durum wheat grown at Fargo and Langdon, North Dakota, in the 1938 crop year, were ex-

[1] These samples were supplied by T. E. Stoa, Chairman of Agronomy, North Dakota Agricultural Experiment Station, and Glenn Smith, Associate Agronomist, United States Department of Agriculture.

perimentally milled and the resultant semolinas processed into macaroni, using the standardized techniques developed by Binnington and Geddes[2] (1936). Langdon is situated in the area noted for durum wheat production, while Fargo is south of this area. Color analyses were also made on the macaroni, as well as various other analytical tests. Determinations of weight, volume, and tenderness of the cooked macaroni, as well as the amount of disintegration, were made at the North Dakota Experiment Station, using the methods described by Binnington, Johannson, and Geddes.

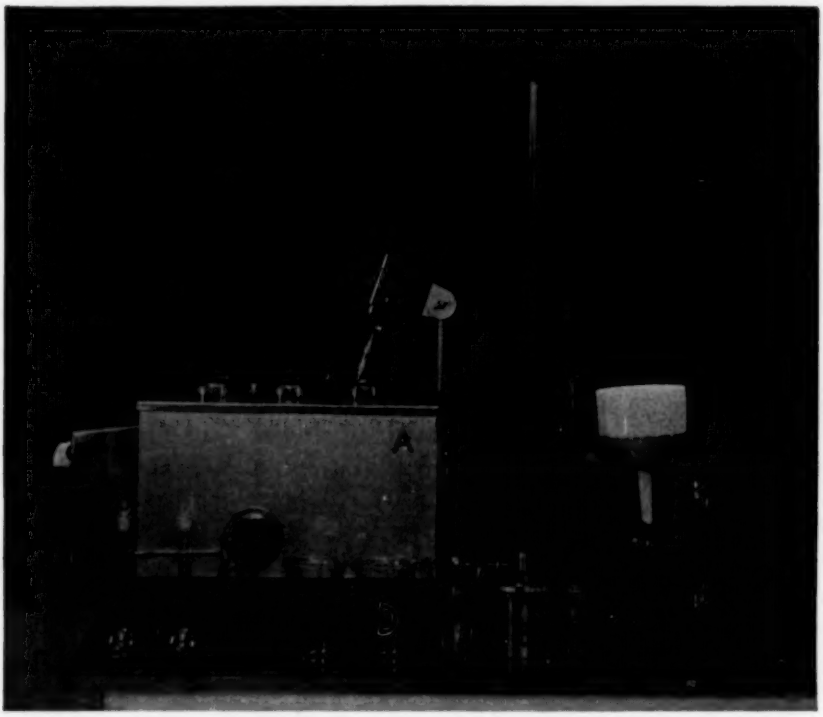

Fig. 1. *A*—Constant-temperature macaroni cooker. *B*—Volumeter used for measuring the increase in volume after cooking. *C*—Volumeter used for measuring the dry volume before cooking. *D*—High-form lipless 500 cc. Pyrex beakers used as containers for the macaroni during cooking.

The macaroni products were cooked in a constant-temperature bath similar to the cooker used by the Canadian laboratory. Prestone was used in the bath rather than water, because of the relatively high temperature employed. A thin layer of mineral oil was added to the Prestone to minimize the evaporation. The temperature was controlled within $\pm 0.5°C$. Six samples were run at one time. A photograph of the apparatus and accessory equipment is shown in Figure 1.

[2] This phase of the experimental work, as well as the analytical tests and color determinations, was carried out by the Dominion Grain Research Laboratory, Winnipeg, Canada, to whom the authors' thanks are due.

The method of procedure was as follows: 500-cc. tall-form lipless beakers were placed in the bath, and 250 cc. of distilled water, previously heated to 95°C., was added to each beaker. When the temperature of the water in the beaker reached 95.5°–96°C., 25 g. of the macaroni product was introduced into the beakers containing the water. The samples were cooked for 30 minutes and stirred at ten-minute intervals throughout the cooking period. Binnington, Johannson and Geddes (1939) had previously established that the 30-minute period was the optimum time of cooking. Further cooking results in excessive softening. At the end of the cooking period the macaroni was drained on a Büchner funnel and washed with boiling distilled water. At the end of a 10-minute draining period the macaroni was transferred to a watch glass and weighed.

The increase in volume was determined by the method described by Binnington, Johannson, and Geddes (1939).

The amount of residue was determined by evaporating the entire amount of the cooking water and washings to dryness and weighing. Previous investigators used aliquots of the cooking water, but, because of the colloidal nature of the liquid and attendant difficulties of securing a representative aliquot, evaporation of the entire quantity seemed expedient.

The method used for determining the tenderness of the cooked macaroni corresponded in essential details with that described by Binnington, Johannson, and Geddes (1939). Load is applied to the sample at a constant rate of approximately 12 g. of mercury per second from a constant head device. The orifice of the delivery tube was adjusted to deliver 57 cc. per minute. The delivery rate was checked quite frequently, as it tends to decrease with surface oxidation. A record was made upon a kymograph drum driven by a small electric motor. A typical record obtained by the use of this instrument and a plan and photograph of the apparatus are shown by Binnington, Johannson, and Geddes. In calculating the tenderness scores, the time required to compress the sample to a thickness of 0.135 inch was used instead of 0.115 inch, as used by those workers. It was necessary to use this value since the lower value of 0.115 did not fall on the linear portion of the curve in all cases. In view of this fact, the tenderness scores obtained are not comparable to the scores reported by Binnington, Johannson, and Geddes. The tenderness tester used in this study was constructed by D. S. Binnington, General Mills Research Laboratory, Minneapolis, Minnesota.

Discussion

Description of the wheats used, semolina yield, and analytical data are shown in Table I. Nine of the samples were grown at Fargo and

TABLE I

WHEAT DESCRIPTION, PROTEIN, ASH, AND PIGMENT DATA[1]

Sample No.	Variety	Semolina yield	Protein		Ash		Pigment	
			Wheat	Semolina	Wheat	Semolina	Wheat	Semolina
		%	%	%	%	%	*ppm.*	*ppm.*
			FARGO					
39–5–13	Kubanka 75	36.6	11.6	10.2	1.63	0.58	5.72	4.53
39–5–1	Kubanka 49	37.5	11.7	10.0	1.74	0.58	6.33	5.35
39–5–11	Kubanka 1440	38.1	11.8	10.4	1.80	0.68	5.81	4.72
39–5–7	Mindum	39.9	12.2	10.8	1.60	0.53	5.78	4.60
39–5–17	Kubanka 314	38.4	12.3	11.1	1.75	0.58	6.31	5.18
39–5–4	Ld 26	38.1	12.5	11.3	1.76	0.57	3.61	2.62
39–5–16	Golden Ball	37.9	13.2	11.3	1.86	0.65	6.15	4.89
39–5–5	Ld 34	40.4	13.2	11.6	1.66	0.62	5.05	4.11
39–5–3	Monad	35.3	13.3	11.4	1.70	0.62	4.42	2.71
Average		38.02	12.42	10.9	1.72	0.60	5.46	4.30
			LANGDON					
39–5–12	Ld 31	36.6	12.0	10.3	1.76	0.64	5.89	4.78
39–5–14	Mindum	36.2	13.0	11.7	1.62	0.69	7.12	5.64
39–5–10	Golden Ball	35.2	13.6	11.6	2.01	0.78	8.56	5.96
39–5–8	R. L. 1183	36.2	13.7	11.9	1.69	0.62	7.61	6.04
39–5–15	Ld 34	36.5	13.7	12.0	1.42	0.60	6.10	4.80
39–5–9	Kubanka 1440	32.5	13.8	12.6	1.83	0.68	7.58	3.65
39–5–2	Monad	33.8	14.0	12.5	1.80	0.63	5.87	3.73
39–5–6	Ld 9 X Mindum	38.1	14.1	12.5	1.72	0.58	6.63	4.67
Average		35.64	13.49	11.89	1.73	0.65	6.92	4.91

[1] Analytical results reported on a 13.5% moisture basis.

eight at Langdon. A number of varieties were included, according to their known characteristics, to cover the range from excellent to poor in macaroni quality. A substantial variation in protein and pigment content is evident among the samples. Although these samples have been arranged in order of increasing wheat protein content, it must be borne in mind that protein content has not been proved to be an important factor in ranking durum wheats for quality, as is the case with bread wheats. The semolina yield, of course, is lower than would have been the case if flour had been produced instead. It will be noticed that the Fargo wheats produced more semolina than the Langdon ones, but were lower in wheat and semolina protein and pigment content. The fact that Langdon durums are higher in pigment content than Fargo durums is in line with evidence already accumulated at this Experiment Station from previous tests.

Table II shows comparative color analyses of the semolina and macaroni produced from the wheats. These values are listed under

TABLE II

COLOR ANALYSES OF SEMOLINA [1] AND MACARONI [2] MADE FROM VARIETIES OF NORTH DAKOTA DURUM WHEAT (1938 CROP)

(Arranged in order of increasing macaroni color score.)

Sample No.	Variety	Hue		Saturation		Brilliance		Single-figure color score	
		Semolina	Macaroni	Semolina	Macaroni	Semolina	Macaroni	Semolina	Macaroni
				FARGO					
39–5–4	Ld 26	24.70	23.34	5.87	5.04	9.09	7.06	16.0	16.7
39–5–16	Golden Ball	24.77	22.72	3.80	5.22	8.83	6.84	10.7	17.3
39–5–11	Kubanka 1440	24.70	23.09	4.19	5.44	8.86	7.03	11.7	17.9
39–5–3	Monad	24.68	22.34	4.55	5.52	8.84	6.82	12.7	18.1
39–5–1	Kubanka 49	24.70	23.32	4.12	5.88	9.02	7.26	11.3	18.9
39–5–7	Mindum	24.77	23.54	4.27	5.96	8.92	7.30	11.9	19.2
39–5–17	Kubanka 314	24.74	23.29	4.05	5.88	8.94	7.06	11.2	19.4
39–5–5	Ld 34	24.72	23.41	4.55	6.28	8.90	7.18	12.6	20.5
39–5–13	Kubanka 75	24.76	23.12	4.46	6.36	8.95	7.06	12.3	20.8
Average		24.73	23.13	4.43	5.73	8.93	7.07	12.3	18.8
				LANGDON					
39–5–10	Golden Ball	24.70	22.42	3.38	5.68	8.74	6.70	9.6	19.0
39–5–2	Monad	24.66	22.38	3 82	5.74	8.73	6.59	10.8	19.5
39–5–9	Kubanka 1440	24.79	22.50	3.54	5.88	8.80	6.68	10.0	19.8
39–5–8	R.L. 1183	24.73	23.03	3.23	6.18	8.78	6.83	9.1	20.8
39–5–6	Ld 9 X Mindum	24.72	23.22	4.38	6.42	8.97	7.03	12.1	21.2
39–5–12	Ld 31	24.62	22.82	3.94	6.44	8.83	6.88	11.0	21.4
39–5–14	Mindum	24.74	22.93	3.46	6.42	8.80	6.89	9.7	21.4
39–5–15	Ld 34	24.77	23.06	4.37	6.82	8.93	7.01	12.1	22.4
Average		24.72	22.80	3.76	6.20	8.82	6.83	10.6	20.7

[1] Data secured with Munsell disks Y-YR 8/6; Y 8/12; N 8/; N/5. Unbalanced illumination N 9.4/; N 8/.

[2] Data secured with Munsell disks YR 6/12; Y 8/12; N 7/; N 4.

three sections: hue, saturation, and brilliance. Binnington and Geddes (1937, 1939) have formulated a color score by combining these three factors in the following manner:

$$\text{Color score} = \frac{\text{Hue}}{\text{Brilliance/Saturation}}$$

The values for hue, brilliance, and saturation were computed according to the formula outlined by Nickerson (1929) and the color score has been found by Binnington, Johannson, and Geddes to give results in close agreement with careful visual classification when working with varietal material. For a further discussion of the color problem reference is made to the papers cited.

It is apparent from the data presented that the Langdon wheats produced macaroni of better and more acceptable color than the Fargo samples. This result is in agreement with conclusions drawn from visual inspection of macaroni produced from wheats grown at these respective locations. The final four wheats in the table, Ld 34, Mindum, Ld 31, and Ld 9 × Mindum, were judged to be entirely satisfactory from the standpoint of color.

It is interesting to note that the color score of Monad grown at Langdon was higher than that of the Mindum grown at Fargo, although it is extremely doubtful if this difference is significant. The variety Ld 26 gave a macaroni having a very undesirable grayish color due, in part at least, to an abnormally low pigment content. Kubanka invariably gives a very pale macaroni, Monad a product possessing a reddish cast, and Golden Ball a macaroni of very unsatisfactory color.

In Table III the comparative cooking quality data obtained from

TABLE III

COMPARATIVE COOKING-QUALITY DATA[1] OBTAINED ON MACARONI PROCESSED FROM THE 1938 CROP

(Arranged in order of increasing weight and volume after cooking.)[2]

Sample No.	Variety	Cooked weight	Increase in weight	Cooked volume	Increase in volume	Residue	Tenderness score
		g.	g.	cc.	cc.	%	
			FARGO				
39–5–16	Golden Ball	350.3	250.3	322.0	252.0	4.5	101.18
39–5–5	Ld 34	354.1	254.1	327.6	257.6	4.8	119.12
39–5–7	Mindum	357.7	257.7	328.4	258.4	4.2	96.67
39–5–3	Monad	358.9	258.9	322.0	262.0	4.8	93.28
39–5–13	Kubanka 75	365.8	265.8	337.8	267.8	4.5	92.52
39–5–4	Ld 26	376.5	276.5	346.8	276.8	4.6	93.42
39–5–1	Kubanka 49	381.4	281.4	353.2	283.2	4.4	97.96
39–5–17	Kubanka 314	382.0	282.0	353.2	283.2	4.3	97.97
39–5–11	Kubanka 1440	382.4	282.4	352.8	282.8	4.0	90.57
Average		367.7	267.7	338.2	269.3	4.4	98.08
			LANGDON				
39–5–9	Kubanka 1440	346.9	246.9	318.4	248.4	3.8	115.37
39–5–8	R. L. 1183	353.5	253.5	325.6	255.6	4.6	100.64
39–5–14	Mindum	355.2	255.2	328.8	258.8	4.5	107.65
39–5–10	Golden Ball	356.2	256.2	327.6	257.6	4.1	116.44
39–5–15	Ld 34	356.7	256.7	328.8	258.8	4.0	102.50
39–5–6	Ld 9 × Mindum	358.9	258.9	330.4	260.4	4.4	128.11
39–5–2	Monad	370.3	270.3	342.8	272.8	4.5	122.13
39–5–12	Ld 31	381.3	281.3	354.8	284.8	4.8	101.79
Average		359.9	259.9	332.2	262.2	4.3	111.83

[1] All results calculated on 100 g. of material containing 13.5% moisture.
[2] A constant dry volume of 70 cc. was obtained.

the macaroni after processing are shown. As the increase in weight and volume are a measurement of the water absorbed during the cooking process, these values are considered to be of primary importance in ranking macaroni for cooking quality.

The values for cooked weight, on the basis of 100 g. of dry material, varied from a minimum of 346.9 g. to a maximum of 382.4 g. These values are somewhat lower than the results reported by Binnington, Johannson, and Geddes (1939). There is a corresponding range in the volumes of the cooked macaroni from 318.4 cc. to 354.8 cc. These

values are also lower than the cooked volumes reported by the Canadian workers.

The average values for both weight and volume of the cooked macaroni samples in this series were higher than similar values of a series of commercial samples [3] reported by Harris and Knowles (1939). This would be expected, as the series of commercially manufactured macaroni contained samples made entirely, or in part, of farina.

A constant dry volume of 70 cc. was obtained on this series of samples. Binnington reported a range in dry volume of 69.6 cc. to 73.2 cc.

The results do not indicate any relationship between color and cooking quality. In a previous study of commercial macaroni the color ranking closely followed the order of cooking quality. This commercial series consisted of macaroni made from varying grades of semolina, mixtures of semolina and farina, and pure farina, and it was found that the macaroni made from the best semolina outranked the other samples both in color and in cooking quality.

The following four varieties appear to be the most satisfactory from the standpoint of cooking quality:

Kubanka 1440—Fargo
Kubanka 314—Fargo
Kubanka 49—Fargo
Ld 31—Langdon

In regard to the tenderness score of the samples, the Langdon series was significantly higher than the Fargo series.

The means, standard deviations, and coefficients of variability computed for the different variables are shown in Table IV, while the correlation coefficients calculated from the data are presented in Table V. It will be noticed that the relationship between cooked weight and cooked volume is very high in each series of samples and justifies the prediction of cooked volume from cooked weight by the following formula, when 25 g. of macaroni product are used:

$$\text{Cooked volume} = -11.34 + 1.0455 \times \text{cooked weight.}$$
$$\text{Error of estimate} = 0.55 \text{ cc.}$$

As the determination of cooked weight is more convenient, rapid, and precise than the determination of cooked volume, the former measurement appears to possess more utility than the determination of volume.

It is also evident that as the protein content increases the tenderness score likewise tends to increase, while the cooked weight tends to decrease. Previous studies at this station have shown that the cooking

[3] The samples were supplied through the courtesy of General Mills, Pillsbury Mills, and Dr. B. R. Jacobs, Director of Research, National Macaroni Manufacturers' Association.

TABLE IV

TABLE OF STATISTICAL CONSTANTS—MEANS, STANDARD DEVIATIONS, AND COEFFICIENTS OF VARIABILITY

	Means	Standard deviations	Coefficients of variability
EXPERIMENTAL SAMPLES [N = 17]			
Cooked weight, g.	91.00	2.993	3.29
Cooked volume, cc.	83.98	2.944	3.50
Wheat protein, %	12.92	0.831	6.43
Semolina protein, %	11.36	0.793	6.98
Wheat ash, %	1.726	0.124	7.16
Semolina ash, %	0.637	0.084	13.16
Tenderness score	104.55	11.174	10.69
COMMERCIAL SAMPLES [N = 18]			
Cooked weight, g.	81.68	5.217	6.39
Cooked volume, cc.	73.89	5.332	7.22
COMBINED EXPERIMENTAL AND COMMERCIAL SAMPLES [N = 35]			
Cooked weight, g.	86.21	6.329	7.34
Cooked volume, cc.	78.80	6.641	8.43

TABLE V

CORRELATION COEFFICIENTS COMPUTED FROM ANALYTICAL AND COOKING QUALITY TEST DATA

Variables correlated			
X	Y	r_{xy}	P[1]
EXPERIMENTAL SAMPLES [N = 17]			
Cooked weight, g.	Cooked volume, cc.	**+.9970**[2]	<.0001
Cooked weight, g.	Semolina protein, %	**−.6227**	.0087
Cooked weight, g.	Tenderness score	−.4174	.0768
Semolina protein, %	Tenderness score	**+.7306**	.0009
Semolina protein, %	Wheat protein, %	**+.9529**	<.0001
Wheat ash, %	Semolina ash, %	+.2901	.2616
COMMERCIAL SAMPLES [N = 18]			
Cooked weight, g.	Cooked volume, cc.	**+.9937**	<.0001
Cooked weight, g.	Macaroni protein, %	**−.7037**	.0011
COMBINED COMMERCIAL AND EXPERIMENTAL SAMPLES [N = 35]			
Cooked weight, g.	Cooked volume, cc.	**+.9966**	<.0001

[1] Probability of the observed correlation coefficient arising from uncorrelated material through errors of random sampling.
[2] Significant correlation coefficients are shown in heavier type.

procedure apparently does not remove appreciable quantities of protein from macaroni, regardless of the type of wheat from which the macaroni is produced. Substantial proportions of the ash, however, were found to be soluble in the cooking water.

The work reported here will later be extended to a more comprehensive study of North Dakota durum wheats, embracing several years' crops. The present study can only be regarded as a preliminary investigation.

Summary and Conclusions

Although a relatively small number of samples were included in the present study, the data obtained would appear to justify the following conclusions:

Durum wheats grown at the Fargo station were higher in semolina yield than wheats grown at Langdon, as a result of differences in test weights.

The Langdon wheats, however, were higher in protein content and produced macaroni of better and more acceptable color. Varietal color differences were also indicated. The "tenderness" score of these samples was also higher than that of the Fargo samples. The latter group of macaroni samples might possibly be classified as definitely "soft" for commercial purposes although the tenderness score limits for commercially acceptable macaroni have not yet been established. There was no relationship between color and cooking quality in this series of samples.

Inasmuch as the determination of cooked weight is more rapid and precise than the determination of cooked volume, the former value is to be preferred, especially as the correlation coefficient between the two variables is sufficiently high to predict the latter variable from the former with a low error of estimate. It was also found unnecessary to determine the dry volume of the macaroni, owing to the relatively constant value obtained for this property.

A negative relationship was evident between cooked weight and protein.

Acknowledgment

The authors are greatly indebted to L. D. Sibbitt,[4] who conducted the milling, processing, and color determinations. Thanks are due to John H. Monge for his valuable technical assistance in obtaining the data presented in this paper.

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THE EFFECT OF ETHYLENE ON FRESHLY HARVESTED WHEAT[1]

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Changes commonly associated with ripening have been shown to occur in several agricultural products after subjecting them briefly to small concentrations of ethylene in air. The effect was first described by Denny (1924) on mature green lemons, where exposure to ethylene, even when diluted 1/1,000,000, resulted in a more active evolution of CO_2 from the fruit and the rapid appearance of yellow color in the skin. Chace and Church (1927) have demonstrated a similar effect on oranges, accompanied by disappearance of free acid and increase in sugar, and Chace and Sorber (1928) have shown that pears rapidly convert starch to sugar and undergo an apparently typical ripening. More recently the view that ethylene produces a natural rather than an artificial ripening has been strengthened by the observation that apples give off ethylene during the natural ripening process (Gane, 1934), which in turn acts on any neighboring green fruit. The effect of ethylene is not confined to fruits, for Chace and Sorber (1936) have shown that walnuts treated therewith are ripened to the extent that the hulls become loose.

In the harvesting of wheat two methods are in common use, the older method whereby wheat is harvested and allowed to remain in the field and the "combine" method, in which the wheat is threshed at the time of cutting and thereafter stored in bins. The after-ripening process sometimes designated as "sweating" is less likely to cause

[1] Food Research Division Contribution No. 472.

trouble when the older method of harvesting is employed than when the wheat is combined. With the former method this biological change largely occurs while the wheat is standing in the shock, whereas with the latter it is delayed, and frequently causes trouble after the wheat has been milled into flour. Swanson (1935) reported that combined wheat is likely to be unsuitable for bread making until it has been aged for some weeks to afford opportunity for after-ripening changes to occur.

The following experiments were conducted with the thought that ethylene might hasten the after-ripening of combined wheat, possibly in such a manner that the grain might become more quickly available for use and the period during which "sweating" takes place might be considerably shortened.

Experimental

Freshly harvested combined wheat (hard spring) was shipped directly from the field to the laboratory, under ice refrigeration. Immediately on arrival the grain, containing 17.1% moisture,[2] was cleaned and stored at −1°. Two days later some of the grain was placed in air containing one part per thousand of ethylene and kept there at room temperature for three days, the air and ethylene being renewed daily. A similar portion of the grain was allowed to stand for three days at room temperature without any treatment. The two samples were then milled and the flour used in regular baking tests.[3] The bread from untreated wheat had smaller volume and was soggy and decidedly greenish in color. That from the ethylene-treated wheat was of excellent texture, color, and apparently in all respects normal (Fig. 1). The treated and untreated samples of the wheat were then both held at room temperature, and several times flour was made from a portion of each and baked as before. The appearance of the bread made after allowing various intervals to elapse between the treatment with ethylene and the milling showed that the untreated wheat gradually attained, after about a month, the characteristics shown after three days by the wheat exposed to ethylene.[4] The data in Table I illustrate this point. Unfortunately, it was not possible to

[2] 17.1% moisture is rather high for combined wheat. The wheat was fully ripe when harvested under moist conditions for experimental work and shipped to us under ice refrigeration. At room temperature, at which our experiments were carried out, the wheat immediately lost this excess moisture and had the appearance of a good hard spring wheat slightly bleached.

[3] Baking formula (standard loaves):

Flour	315 g.
Salt	5.5 g.
Yeast	8.0 g.
Sugar	20.0 g.
Shortening	6.5 g.
Water	170 cc.

[4] The improvement of wheat by treatment with ethylene had been previously demonstrated by us with some wheat we obtained through the Foreign Agricultural Service from Argentina during March, 1939. However, the wheat was several weeks old by the time we received it and the results of the experiments were not so striking. The flour from the ethylene-treated wheat made decidedly better bread than the controls of untreated wheat.

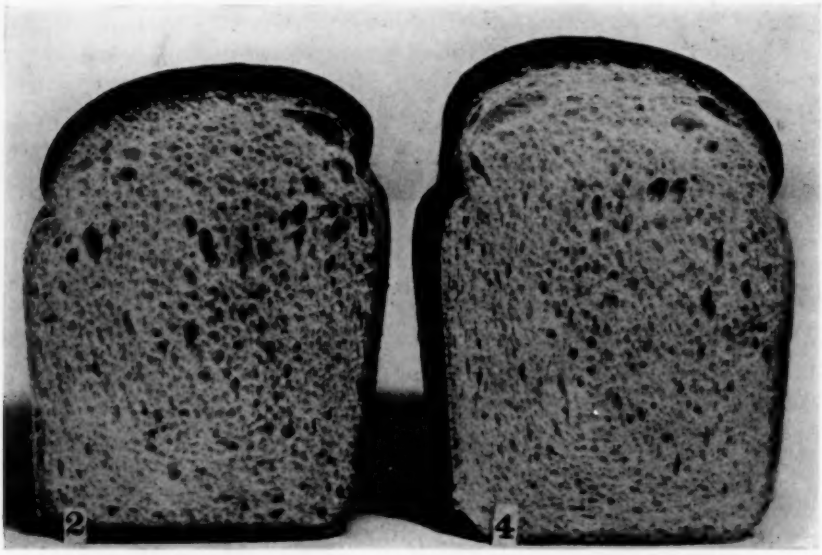

Fig. 1. Effect of ethylene on wheat: No. 2, untreated; No. 4, treated—after 19 days.

make more frequent baking tests, so these data are admittedly scanty, but the difference between the treated and untreated wheat was so striking at first as to leave no doubt of the general trend of the results.

TABLE I

EFFECT OF ETHYLENE TREATMENT ON VOLUME OF LOAF

When this experiment was begun the wheat had been harvested seven days and had been kept near 0°C. in the meantime.

Date	Time since harvest	Loaf volume (cold)		Remarks	
		Treated	Control	Treated	Control
	Days	*cc.*	*cc.*		
8– 3–39	7	—	2025	——	Green, coarse texture.
8– 8–39	12	2140	2070	Light in color, fine grain & texture.	Green, coarse texture.
8–15–39	19	2335	2200	Better grain & texture, color same.	Some improvement over original.
9–28–39	60	—	2225	——	Normal loaf of bread.

The influence of ethylene in improving the quality of the bread is evidently exerted on the grain, and not on the flour, as evidenced by the observation that the flour from untreated wheat gave not only a poor bread, as stated, but continued to do so after the milled flour (patent) had been exposed to 1/1,000 ethylene, in the same way as the wheat was treated. Such flour does improve somewhat with age, as does flour from the untreated wheat, but no particular differences in

behavior were noticeable. Bailey and Fifield (1939) obtained similar results on the treatment of flour with ethylene.

Experiments which it was thought might show an increased rate of metabolism in the treated wheat failed to indicate this. Thermos jugs holding about 2.5 pounds of wheat were filled with treated and untreated specimens and the rise in temperature of each was observed over a twenty day period. No particular difference was noted, however. Furthermore, oxygen-absorption measurements made in a Warburg apparatus showed no difference between the rates of oxygen consumption by one gram of treated and one gram of untreated wheat. Experiments with whole grains are subject, however, to so many errors that in the absence of very great differences between experiment and control nothing definite can be said.

On the other hand, a marked change in the viability of the wheat, as evidenced by the usual germination test, was caused by the ethylene treatment. Green wheat, as obtained from the combine harvester, is known to give low germination values (Atkinson and Jahnke, 1918; Swanson, 1938). When treated with ethylene in the proportion of one part per thousand of air on three successive days as described before, the wheat appeared to be stimulated slightly for a few days after treatment, but thereafter lost viability on continued storage instead of gaining it, as did the control. After a single treatment with a 1/10,000 ethylene-air mixture, however, an increase in viability was observed. Unfortunately the wheat was by this time past the stage where there was much difference in bread-making quality between the treated and untreated portions. Nevertheless, the effect of ethylene on the germination was marked. The wheat behaved as though capacity for germination was increased by exposure to ethylene, but it appeared that too much ethylene was decidedly harmful in this connection. The germination tests run at 20° on 100 grains are shown in Table II as the percentage of sprouted grains in six days.

TABLE II

GERMINATION TESTS—PERCENT GERMINATION IN SIX DAYS AT 20°

Treatment of wheat stored at room temperature	Days stored after ethylene treatment						
	0	1	2	3	4	8	23
None	50	54	48	54	56	—	—
Stored 3 days in ethylene, 1/1,000	54	52	86	68	64	—	76
Stored 3 days in ethylene, 1/10,000	62	58	74	74	—	74	—

By the time these experiments had been completed, the wheat kept in cold storage no longer behaved differently from normal wheat or that kept continuously at room temperature, so no further experiments

appear possible until a new crop is available. Whether such a process would be of practical value in handling wheat is not in the province of this laboratory to say. In any case experiments on a far greater scale would be required to demonstrate its utility. The present publication is made only as a suggestion and a matter of record.

Summary

A necessarily brief experiment on freshly harvested combine wheat has shown that small doses of ethylene are able to mature such wheat very quickly, in the sense that it becomes suitable for bread making. No reliable data pointing to an accelerated metabolism were obtained, although it seems probable that it occurs. An immediate increase in the viability of the treated wheat was observed with a low concentration of ethylene, but there is evidence that more ethylene is decidedly harmful.

The implications of these observations may be of sufficient importance to warrant attention by chemists better equipped to study the question. If the ethylene treatment will serve as a means of hastening the well known after-ripening process popularly designated as "sweating" its possibilities of use in industrial processing are worthy of consideration.

Acknowledgment

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BARLEY AND MALT STUDIES. VI. EXPERIMENTAL MALTING OF BARLEYS GROWN IN 1938[1]

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(Read at the Annual Meeting, May 1939)

The regional investigations on the malting quality of the five standard barley varieties grown in 1938 are presented as a progress report. The study of the five varieties, Oderbrucker, Wisconsin Barbless, Velvet, Manchuria, and Trebi, has been continued in cooperation with the various states for five seasons (1934 to 1938) and has been terminated by action of the Barley Improvement Council with the study of the 1938 crop. The detailed data on the physical and chemical factors of the barleys and malts for the five standard varieties and the other barley varieties grown at the various stations during the five-year period will be presented in a later publication. However, a summary of the varietal and yearly data, and a brief discussion of the data are presented in this paper.

Growing and Harvesting Conditions During the Season of 1938

Growing conditions were relatively favorable for barley development during the 1938 growing season. Soil moisture was sufficiently abundant at most of the stations to give comparatively normal growth. Continued rainfall into the ripening and harvest period, however, damaged the quality of the barley at some of the stations. The damage caused by lodging of the grain, blight disease, and high moisture content of the grain while in the shock was more severe at the stations in the central section of the spring barley area than at the western stations of this area.

In many respects, the 1938 season was similar to that of 1935. In the 1935 season, however, there was a moisture shortage during the latter part of the growing season which materially reduced the quality of the barleys grown at Lincoln, Nebr., Brookings, S. D., and Fargo, N. D. In contrast, in 1938 the moisture conditions were very favorable for barley production at these western stations, which resulted in a more uniform quality of barley at all stations than in any of the previous seasons included in the investigations.

[1] Based on cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and the Wisconsin Agricultural Experiment Station. The cooperative investigations include the agricultural experiment stations of California, Colorado, Illinois, Iowa, Michigan, Minnesota, Montana, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin, where the uniform barley varietal series have been grown each year. The Federal WPA has contributed to the research program during the past year through a grant under the University of Wisconsin WPA Natural Science Project. The United States Maltsters Association has cooperated through an industrial fellowship grant to the University of Wisconsin.

Comparison of the Barleys and Malts from the Thirteen Stations

Thirteen locations provided barley of the five standard varieties grown in 1938. The barleys were malted, including malting controls, under approximately the same conditions as in previous years. The barleys were steeped to a moisture content of 46% and malted at 46% to 44% moisture. The average steep moisture for all barleys was 45.8% and the average moisture content of the green malts at the end of the germination period was 44.6%. The barleys and malts were analyzed for the physical and chemical factors as in the previous years. The detailed data for the physical and chemical factors of the barleys and malts are given in Table I. The discussion will be limited to some of the more important characteristics of the 1938 season's barleys and malts.

The grain yields in bushels per acre varied widely for the different stations. At the stations in the central area barley yielded about the same as the average for the five-year period. Yields at Brookings, S.D., and Lincoln, Nebr., were higher than in the previous years at these stations. The yields reported for Davis, Calif., are low for all varieties except Trebi because of shattering of kernels before the barley was mature. Likewise, Velvet was low at Fort Lewis, Colo., as a result of shattering and insect injury.

The barleys from all of the stations were relatively plump and mature. The barleys from Lincoln, Nebr., Brookings, S. D., and Fargo, N. D., were superior in quality to those of any of the previous seasons except perhaps the barleys grown at these stations in 1934. The barleys grown at Waseca, Minn., and Madison, Wis., for the most part were not equal in quality to those grown at these locations in 1935. The barleys from East Lansing, Mich., and Urbana, Ill., were similar in quality to those of the three previous seasons. The varieties produced at DeKalb, Ill., were more like those grown in 1936, in quality. And, finally, the barleys from Bozeman, Mont., Fort Collins and Fort Lewis, Colo., and Davis, Calif., were, in general, similar in quality to those of the previous season's crops.

The bushel weight of the barleys varied considerably at the different stations. The bushel weights of all the barleys grown at Madison, Wis., and of Velvet and Trebi grown at East Lansing, Mich., were abnormally low, largely because of poor threshing and partly germinated kernels, rather than a large amount of thin barley. These conditions were responsible for the similarity of bushel weight of barley and malt in the lots mentioned above, as the cleaning of the malt removed the awns and rootlets and loose hulls that were instrumental in lowering the bushel weight of barley.

TABLE I

THE PHYSICAL AND CHEMICAL FACTORS OF BARLEYS AND MALTS FOR THE FIVE STANDARD VARIETIES GROWN AT THE VARIOUS STATIONS IN 1938

Location and variety	Yield of barley per acre	Bushel weight of barley, dry basis	Kernel weight of barley, dry basis	Total protein in barley, N ×6.25	Ash content of barley	Hull content of barley	Moisture content steeped barley	Moisture content green malt	Recovery of malt from barley, dry basis	Bushel weight of malt, 4% basis	Kernel weight of malt, dry basis	Moisture content malt	Extract fine grind, dry basis	Time conversion	Diastatic power	Total nitrogen in malt	Total protein in malt, N ×6.25	Soluble nitrogen in wort	Soluble nitrogen in relation to malt nitrogen	Formol nitrogen relation to malt nitrogen
Oderbrucker, Ped. 5-1	*Bu.*	*Lbs.*	*Mg.*	%	%	%	%	%	%	*Lbs.*	*Mg.*	%	%	*Min.*	°*L.*	%	%	%	%	%
East Lansing, Mich.	—	38.6	29.9	13.06	2.91	13.6	45.1	46.5	85.7	35.9	26.3	5.1	73.6	5	183	2.13	13.31	.708	33.2	6.3
Urbana, Ill.	33.7	39.3	29.3	11.58	3.05	15.0	46.5	44.6	87.3	35.7	26.6	5.0	75.5	7	154	1.84	11.52	.731	39.7	8.0
DeKalb, Ill.	30.5	38.5	27.7	11.70	3.26	12.6	46.3	44.5	87.4	36.2	24.4	5.2	76.1	7	168	1.81	11.32	.779	43.0	8.5
Madison, Wis.	17.7	33.6	24.8	14.61	3.36	13.9	46.5	43.4	90.0	33.9	22.6	4.9	72.7	<5	238	2.42	15.12	.895	37.0	7.4
Waseca, Minn.	43.7	38.6	25.4	13.72	3.39	12.5	47.0	45.2	85.8	34.7	23.0	5.7	73.1	7	148	2.12	13.25	.771	36.4	6.9
Kanawha, Iowa	24.8	37.8	23.5	13.67	3.82	16.3	47.6	45.3	86.5	34.4	21.1	4.9	73.4	<5	208	2.32	14.50	.906	39.1	8.4
Fargo, N. D.	30.3	39.0	23.2	15.94	3.50	14.5	45.7	48.7	84.6	32.3	20.2	5.8	72.0	<5	240	2.63	16.44	.897	34.1	6.5
Brookings, S. D.	30.4	40.0	25.6	17.50	3.02	13.6	45.9	45.0	86.7	35.0	21.9	5.2	72.3	<5	270	2.97	18.56	.980	32.9	6.3
Lincoln, Neb.	21.5	41.3	25.1	16.36	2.91	12.9	45.7	45.5	86.4	35.9	21.9	5.2	71.5	5-7	249	2.62	16.38	.861	32.9	6.6
Bozeman, Mont.	58.8	47.0	36.3	15.25	2.84	10.7	44.8	45.0	86.5	39.3	31.7	5.8	75.3	<5	256	2.52	15.75	.891	35.4	7.1
Fort Collins, Colo.	49.6	46.5	32.5	13.97	2.81	10.6	44.6	44.9	85.8	38.5	27.8	6.0	76.5	<5	224	2.39	14.94	.799	33.4	6.4
Fort Lewis, Colo.	50.9	41.3	32.1	12.78	3.19	11.0	45.6	44.3	85.7	40.8	28.6	5.8	76.4	5	182	2.09	13.06	.701	33.5	6.7
Davis, Calif.	12.5*	44.1	32.0	14.63	2.94	11.7	44.0	41.1	89.2	40.3	29.8	5.5	73.2	5	170	2.44	15.25	.687	28.2	5.6
Average		40.4	28.3	14.21	3.15	13.0	45.8	44.9	86.7	36.4	25.1	5.4	74.0	5.1	224	2.33	14.57	.816	35.2	7.0
Wisconsin Barbless Ped. 38																				
East Lansing, Mich.	—	38.8	29.6	12.22	2.79	14.5	46.4	45.2	87.1	36.4	27.2	5.5	72.8	7-10	137	2.10	13.13	.542	25.8	4.8
Urbana, Ill.	27.4	38.2	31.3	11.90	3.09	15.6	46.3	44.3	87.9	36.0	26.1	5.2	73.1	7	129	1.91	11.96	.619	32.4	6.0
DeKalb, Ill.	34.8	38.2	26.5	11.46	3.12	14.4	45.9	44.1	87.5	35.9	24.3	5.1	74.3	7	119	1.74	10.88	.661	38 0	6.9
Madison, Wis.	32.9	35.0	28.1	14.36	3.18	14.8	45.2	42.8	89.3	35.0	23 7	5.2	70.4	7	151	2.39	14.94	.671	28.1	7.0
Waseca, Minn.	56.8	38.6	25.7	12.95	3.24	14.1	46.9	45.0	87.2	35.7	22.7	5.8	72.8	7	154	2.18	13.63	.648	29.7	4.9
Kanawha, Ia.	39.9	39.4	24.9	12.95	3.70	16.2	45.9	45.5	86.8	35.5	22.7	5.7	72.7	7	144	2.15	13.44	.679	31.6	5.4
Fargo, N. D.	46.5	40.4	27.2	14.78	3.29	13.5	45.7	45.1	87.2	35.8	23.7	5.5	70.9	7	147	2.44	15.25	.610	25.0	4.4
Brookings, S. D.	49.5	41.2	28.6	15.38	2.92	12.3	44.4	44.6	85.2	37.0	24.9	5.6	72.9	5	160	2.54	15.86	.663	26.1	5.1
Lincoln, Neb.	38.8	41.2	25.0	14.43	2.86	16.0	45.2	45.4	85.0	35.8	22.5	5.9	71.6	7	164	2.38	14.88	.679	28.5	4.9
Bozeman, Mont.	70.0	46.7	38.7	14.66	2.80	12.5	44.4	44.6	86.9	40.6	34.8	6.3	73.9	5	181	2.38	14.88	.587	24.7	4.5
Fort Collins, Colo.	67.7	45.4	32.0	15.25	2.80	11.5	44.4	44.8	87.2	39.3	27.7	6.1	73.4	5	158	2.45	15.31	.646	26.4	4.9
Fort Lewis, Colo.	77.7	43.5	33.1	11.88	3.02	11.9	45.6	44.4	89.2	38.9	30.3	5.7	74.8	10	111	1.77	11.08	.491	27.7	4.9
Davis, Calif.	17.8*	43.5	33.7	13.06	2.84	12.2	45.9	43.7	87.0	41.0	29.2	5.8	75.2	10	127	2.19	13.69	.577	26.3	4.3
Average		40.8	29.6	13.48	3.05	13.8	45.6	44.6	87.2	37.1	26.1	5.6	73.0	7.1	145	2.20	13.76	.621	28.5	5.2

* Low yields due to the kernels dropping from heads before harvest.

TABLE I—*Continued*

Location and variety	Yield of barley per acre	Bushel weight of barley, dry basis	Kernel weight of barley, dry basis	Total protein in barley, N ×6.25	Ash content of barley	Hull content of barley	Moisture content steeped barley	Moisture content green malt	Recovery of malt from barley, dry basis	Bushel weight of malt, 4% basis	Kernel weight of malt, dry basis	Moisture content malt	Extract fine grind, dry basis	Time conversion	Diastatic power	Total nitrogen in malt	Total protein in malt, N ×6.25	Soluble nitrogen in wort	Soluble nitrogen in relation to malt nitrogen	Formol nitrogen relation to malt nitrogen
Velvet, Minn. 447	*Bu.*	*Lbs.*	*Mg.*	%	%	%	%	%	%	*Lbs.*	*Mg.*	%	%	*Min.*	°*L.*	%	%	%	%	%
East Lansing, Mich.	—	35.7	29.7	12.44	3.05	14.8	45.6	45.4	85.8	34.8	25.9	5.3	74.4	5	146	2.04	12.75	.717	35.1	6.6
Urbana, Ill.	27.0	38.9	28.4	12.13	3.05	14.9	45.9	43.9	88.0	35.6	24.5	5.1	74.8	5	150	1.91	11.96	.770	40.3	8.0
DeKalb, Ill.	29.7	38.9	26.2	12.02	3.30	13.2	46.3	44.1	88.4	36.0	24.0	5.1	76.2	5	136	1.90	11.90	.792	41.7	8.0
Madison, Wis.	23.8	34.4	23.1	14.73	3.18	15.4	47.3	41.8	91.1	34.5	21.2	5.0	71.6	<5	183	2.47	15.44	.885	35.8	7.0
Waseca, Minn.	47.7	39.0	23.5	13.32	3.64	15.2	46.7	44.6	87.8	36.0	20.2	5.6	73.1	<5	200	2.24	14.00	.842	37.6	8.0
Kanawha, Ia.	39.8	39.1	23.3	13.38	3.70	14.4	47.0	45.1	88.3	34.0	20.7	5.1	72.3	5	205	2.17	13.56	.846	39.0	8.4
Fargo, N. D.	38.9	42.7	25.4	15.16	3.29	13.2	43.6	45.2	86.2	36.5	21.7	5.2	72.0	<5	208	2.52	15.75	.841	33.4	7.0
Brookings, S. D.	34.9	40.1	23.7	15.91	3.14	16.5	45.4	44.7	85.5	36.1	21.1	5.3	74.4	5	215	2.72	17.06	.898	33.0	6.7
Lincoln, Neb.	24.8	42.3	24.8	15.56	2.94	16.7	46.3	45.8	84.8	36.3	21.3	5.7	72.8	<5	221	2.60	16.25	.893	34.3	6.4
Bozeman, Mont.	73.2	47.5	35.4	15.25	2.92	10.4	44.0	44.8	86.7	39.7	31.3	6.2	75.7	<5	231	2.50	15.63	.799	32.0	6.2
Fort Collins, Colo.	59.4	44.0	31.6	16.00	2.96	11.4	45.0	44.9	87.6	36.6	27.4	5.7	73.8	<5	243	2.57	16.06	.866	33.7	6.6
Fort Lewis, Colo.	36.4*	40.5	32.2	12.06	3.06	12.6	45.6	44.6	87.1	38.6	28.2	5.9	76.6	5	139	1.94	12.13	.713	36.8	7.4
Davis, Calif.	13.9*	43.6	31.7	13.38	2.98	14.3	44.0	42.3	89.3	39.3	29.2	5.0	73.7	5	164	2.20	13.72	.677	30.8	6.2
Average		40.5	27.6	13.95	3.17	14.1	45.6	44.4	87.4	36.5	24.4	5.4	74.0	5.0	188	2.29	14.32	.811	35.7	7.1
Manchuria, N.D. 2121																				
East Lansing, Mich.	—	38.5	29.7	13.72	3.00	11.8	48.5	45.9	87.1	35.7	26.3	5.6	74.1	<5	212	2.30	14.36	.823	35.8	7.5
Urbana, Ill.	25.0	37.6	27.3	13.19	3.11	11.7	45.6	44.7	87.7	34.8	24.0	4.9	74.3	<5	169	2.10	13.13	.852	40.6	8.1
DeKalb, Ill.	30.0	37.4	26.4	12.02	3.36	12.8	46.3	44.1	87.9	34.7	23.1	5.0	75.1	<5	158	1.98	12.40	.856	43.2	9.3
Madison, Wis.	19.0	32.6	23.4	15.25	3.17	14.2	47.3	43.6	91.0	32.3	20.4	5.0	72.5	<5	257	2.59	16.19	.991	38.3	8.0
Waseca, Minn.	41.5	36.9	23.2	13.86	3.56	15.9	47.7	45.3	86.7	33.5	20.9	5.0	73.7	<5	209	2.31	14.43	.975	42.2	9.4
Kanawha, Ia.	26.1	37.8	23.0	13.86	3.69	13.9	47.6	45.5	86.7	34.6	20.6	5.2	73.0	5	223	2.24	13.99	.882	39.4	8.3
Fargo, N. D.	46.4	42.3	25.4	13.94	3.06	12.0	45.1	44.7	87.1	36.1	22.8	5.1	74.3	7	205	2.31	14.44	.740	32.0	6.1
Brookings, S. D.	28.2	39.5	23.7	16.34	2.91	11.9	45.2	44.3	86.9	35.0	20.9	5.3	73.4	5	241	2.76	17.24	.915	33.1	6.8
Lincoln, Neb.	24.0	40.9	22.6	15.75	2.95	12.9	45.7	45.5	86.6	35.2	20.8	5.0	72.2	<5	244	2.54	15.88	.906	35.7	7.3
Bozeman, Mont.	66.1	46.5	35.6	14.97	2.86	9.7	44.4	45.6	85.8	38.3	30.1	6.3	75.9	<5	248	2.49	15.56	.866	34.8	6.9
Fort Collins, Colo.	54.2	44.9	29.4	14.59	2.81	13.5	45.4	45.0	87.1	37.4	25.8	5.4	75.9	<5	238	2.46	15.38	.866	35.2	6.9
Fort Lewis, Colo.	53.6	41.6	31.1	13.06	3.16	13.8	45.6	45.4	87.2	37.9	27.4	5.4	76.4	5	183	2.12	13.25	.773	36.5	7.1
Davis, Calif.	18.0*	44.8	32.0	13.56	2.80	10.3	44.0	41.7	90.6	40.9	30.1	5.7	75.0	5	164	2.20	13.72	.654	29.7	5.8
Average		40.1	27.1	14.16	3.11	12.6	46.0	44.7	87.6	35.9	24.1	5.3	74.3	4.5	212	2.36	14.73	.854	36.4	7.5

TABLE I—*Continued*

Location and variety	Yield of barley per acre	Bushel weight of barley, dry basis	Kernel weight of barley, dry basis	Total protein in barley, N ×6.25	Ash content of barley	Hull content of barley	Moisture content steeped barley	Moisture content green malt	Recovery of malt from barley, dry basis	Bushel weight of malt, 4% basis	Kernel weight of malt, dry basis	Moisture content malt	Extract fine grind, dry basis	Time conversion	Diastatic power	Total nitrogen in malt	Total protein in malt, N ×6.25	Soluble nitrogen in wort	Soluble nitrogen in relation to malt nitrogen	Formol nitrogen relation to malt nitrogen
Trebi	*Bu.*	*Lbs.*	*Mg.*	%	%	%	%	%	%	*Lbs.*	*Mg.*	%	%	*Min*	°*L.*	%	%	%	%	%
East Lansing, Mich.	—	35.8	36.6	11.75	3.04	13.7	46.1	44.0	88.9	35.8	33.9	5.6	74.6	10	150	1.96	12.25	.596	30.4	5.8
Urbana, Ill.	33.0	38.9	32.7	13.56	3.12	13.2	46.7	44.3	87.8	35.5	29.0	5.2	73.1	7	124	2.11	13.19	.745	35.3	7.0
DeKalb, Ill.	40.3	39.4	37.7	9.33	3.10	13.2	45.7	44.2	88.4	36.9	33.5	5.4	78.8	10	108	1.50	9.38	.620	41.3	8.6
Madison, Wis.	27.9	31.9	31.2	14.25	2.87	14.9	47.7	42.7	91.6	33.5	27.6	5.2	70.8	5	178	2.37	14.81	.738	31.1	5.8
Waseca, Minn.	50.3	35.7	30.5	11.69	3.47	17.6	47.7	44.8	87.7	34.1	26.7	5.7	73.3	7	167	2.02	12.63	.675	33.4	6.4
Kanawha, Ia.	34.6	37.7	30.4	12.51	3.58	16.9	46.8	44.9	88.0	34.2	28.1	5.7	73.8	7	154	1.94	12.13	.677	34.9	6.2
Fargo, N. D.	53.5	41.7	33.8	15.06	2.93	13.6	45.6	44.2	89.7	36.8	30.4	5.4	72.9	10	194	2.44	15.25	.618	25.3	4.3
Brookings, S. D.	41.2	40.3	32.8	14.59	2.54	14.7	45.9	44.1	88.9	33.9	29.1	5.6	73.2	5	177	2.44	15.25	.695	28.5	5.5
Lincoln, Neb.	41.6	38.7	28.2	15.61	2.91	17.9	45.4	45.2	87.0	36.0	24.6	5.4	69.6	5	217	2.64	16.50	.697	26.4	4.7
Bozeman, Mont.	100.8	46.3	45.4	14.03	2.85	10.2	44.0	43.9	89.5	40.2	40.8	6.2	75.7	7	211	2.30	14.38	.564	24.5	4.1
Fort Collins, Colo.	67.4	44.6	39.6	11.66	2.51	13.9	44.8	43.5	89.8	38.4	36.3	6.1	78.0	7–10	161	1.95	12.19	.587	30.1	4.9
Fort Lewis, Colo.	85.2	44.0	43.3	10.66	2.95	11.7	45.6	44.6	89.6	40.3	39.1	6.2	78.1	20	144	1.78	11.13	.461	25.9	4.1
Davis, Calif.	60.6	41.0	37.2	12.56	2.80	14.6	45.6	43.7	87.0	38.8	33.2	5.8	74.2	20	124	2.17	13.56	.527	24.3	4.4
Average		39.7	35.3	12.87	2.97	14.3	46.0	44.2	88.8	36.5	31.7	5.7	74.3	9.3	162	2.12	13.28	.631	30.1	5.5
Grand Average		40.3	29.6	13.73	3.09	13.6	45.8	44.6	87.5	36.5	26.3	5.5	73.9	6.1	188	2.26	14.13	.746	33.2	6.5

The kernel weight of the barleys was relatively high for all five varieties. The varietal ranking for the average kernel weight of barley from all stations was the same as in the previous years. The four varieties, Wisconsin Barbless, Oderbrucker, Velvet, and Manchuria, showed some shifting in rank at the individual stations.

The protein content of the barleys varied widely between the central and western stations of the spring barley area. The protein content of the barleys grown at Madison was relatively high. The barleys from Fargo, Brookings, Lincoln, and Bozeman were all high in protein content in contrast to those from the other stations. Protein content varied from 9.3% for Trebi grown at DeKalb to 17.5% for Oderbrucker grown at Brookings.

Ash content of the barleys was relatively high and fairly uniform at all stations. The varieties were very similar in ash content; the maximum variation in average ash content for the five varieties was 0.2%.

Hull content of the barleys showed considerable variation at the different stations. In general, the average hull content at the stations in the central area was slightly higher than the five-year average, whereas the barleys from Brookings, Lincoln, and Fargo were below the average at these stations.

Recovery of malt from barley showed a difference in varietal trend from the previous years. The average recovery from the Wisconsin Barbless variety in 1938 was not greatly different from that of Oderbrucker, Velvet, and Manchuria, whereas, in the previous years recovery was higher for this variety than for the other three. Trebi averaged highest in recovery as in the previous years except 1935, when Wisconsin Barbless exceeded it.

The extract content of the malts was comparatively high except for the barleys grown at Madison and Lincoln. The varietal ranking for the average extract content was the same as in the previous years. There was some shifting in the varietal ranking at the individual stations; however, Wisconsin Barbless malts were the lowest in extract content, while the Manchuria and Trebi malts were highest.

The diastatic power of the malts was relatively high for all of the varieties and at most of the stations. The malt lowest in diastatic power, 108° L., was from Trebi from DeKalb. The highest, 270° L., was from Oderbrucker grown at Brookings. These two barleys were likewise the lowest and highest, respectively, in protein content. The varieties ranked the same for the average diastatic power as in the previous years. There was also less shifting in rank between the varieties at the individual stations than for many other factors.

The conversion time on the malts was relatively rapid. The average conversion time was similar to that of 1935.

The soluble nitrogen in the wort as well as the soluble nitrogen as percentage of malt nitrogen was relatively high for the malts from most of the stations. The varietal ranking for average soluble nitrogen in the wort was similar to the previous year. There was as usual a marked difference between varieties in the soluble nitrogen in the wort as percentage of malt nitrogen. Permanently soluble nitrogen and formol nitrogen expressed as the percentage of the malt nitrogen showed variations similar to soluble nitrogen in the wort for both varieties and stations.

The malts from the five standard varieties grown at the 13 locations showed less variation in quality between stations than in any of the previous years studied. The varieties have maintained essentially the same ranking based on the average for all stations as in the previous years for the major characteristics studied. There was, however, greater fluctuation in the rank of the varieties at the individual stations than in the previous year.

Five-Year Comparison of the Barleys and Malts from Six Stations

The comparative averages for the barleys and malts from the six stations which have supplied barley of the five standard varieties for each of the five years show the reaction of the varieties for the five-year period and the influence of seasonal conditions on these barleys. The six stations from which the data are included in the average are: (1) East Lansing, Mich., (2) Madison, Wis., (3) Waseca, Minn., (4) Kanawha, Iowa, four years, Emmetsburg, Iowa, one year, (5) Brookings, S. D., and (6) Bozeman, Mont. The varietal averages for the five years (1934–38) and the averages for the five varieties combined for each year are given in Table II. The analysis of variance of some physical and chemical factors of barleys and malts for the same varieties and stations for the five-year period are given in Table III.

The average bushel weights of barley were very similar for the four varieties, while that for Trebi was lower. The bushel weights varied considerably between the individual stations. The variation between stations and between years was much greater than the variation between varieties. However, the varieties held the same position at each of the locations.

The average kernel weight of barley showed a varietal difference for the five-year average at the six stations. The varieties had approximately the same ranking as in the average for the larger number of stations for each year. The yearly averages of all five varieties showed a higher kernel weight of barley at these six stations in 1934,

TABLE II

AVERAGES FOR THE FIVE STANDARD VARIETIES FOR THE FIVE YEARS 1934 TO 1938 AND YEARLY AVERAGES FOR THE FIVE VARIETIES COMBINED FOR THE BARLEYS GROWN AT THE SIX STATIONS: EAST LANSING, MICH., MADISON, WIS., WASECA, MINN., KANAWHA AND EMMETSBURG, IOWA, BROOKINGS, S. D., AND BOZEMAN, MONT.

	Varietal average for five years (1934–38)					Yearly averages for five varieties				
	Oderbrucker	Wisconsin Barbless	Velvet	Manchuria	Trebi	1934	1935	1936	1937	1938
Bushel weight barley, dry basis, *lbs.*	39.6	39.7	39.9	39.3	38.7	39.4	41.1	40.0	37.8	39.0
Kernel weight Barley, dry basis, *mg.*	26.1	27.7	25.5	24.9	33.7	29.8	27.0	26.3	26.1	28.7
Hull content barley, dry basis, %	13.8	14.0	14.2	12.8	13.9	12.5	10.9	13.3	15.8	13.7
Recovery malt from barley dry basis, %	87.0	88.6	88.1	87.1	89.5	87.8	86.4	89.2	89.2	87.6
Bushel weight malt, 4% basis, *lbs.*	35.9	36.7	36.6	35.7	35.7	36.1	37.4	35.3	34.2	35.7
Kernel weight malt, dry basis, *mg.*	23.1	26.8	22.6	22.0	30.6	27.1	23.5	23.5	23.5	25.7
Extract content, dry basis, %	72.3	70.5	71.8	72.8	72.5	71.6	72.7	70.8	71.2	73.4
Total protein in malt, %	15.5	14.5	14.8	15.3	13.9	16.5	14.3	14.3	14.2	14.6
Soluble nitrogen in wort as protein, %	5.4	4.0	5.0	5.5	4.1	5.0	5.0	4.6	4.6	4.9
Soluble nitrogen in relation to malt nitrogen, %	34.7	27.5	34.6	35.9	29.4	29.7	35.0	31.6	32.6	33.2
Conversion time, *min.*	5.4	8.3	5.6	5.5	10.0	7.7	5.9	6.2	9.6	5.4
Diastatic power, °*L*	102	124	153	191	145	109	164	168	168	195

TABLE III

ANALYSIS OF VARIANCE (F VALUES) OF PHYSICAL AND CHEMICAL FACTORS OF BARLEYS AND MALTS FOR THE FIVE STANDARD [1] VARIETIES GROWN AT SIX STATIONS [2] FOR THE FIVE YEARS 1934 TO 1938

Source	Deg. freedom	Bushel weight of barley	Kernel weight of barley	Estimated time to reach 46% moisture in steep	Recovery of malt from barley	Extract in malt, dry basis	Protein in malt	Soluble nitrogen in wort	Soluble nitrogen as per cent of malt nitrogen	Conversion time in mashing	Diastatic power of malt	5% point	1% point
Varieties	4	5.4	226.9	13.1	12.9	12.4	22.7	128.5	59.1	20.6	70.0	2.49	3.56
Years	4	337.4	46.0	51.3	16.6	17.5	65.7	53.7	23.3	3.2	78.2	2.49	3.56
Stations	5	37.4	261.0	8.3	3.7	36.1	52.9	8.9	13.4	13.4	27.5	2.33	3.26
Var. × Sta.	20	1.3	0.7	1.2	0.5	0.5	1.7	1.7	0.4	1.1	1.6	1.73	2.16
Var. × Year	16	2.6	1.28	1.2	1.0	1.0	2.9	1.8	0.7	1.4	2.4	1.81	2.29
Sta. × Year	20	31.2	29.2	13.2	7.2	11.5	24.6	12.3	2.6	4.3	9.8	1.73	2.16
Error	80	—	—	—	—	—	—	—	—	—	—	—	—
Total	149	—	—	—	—	—	—	—	—	—	—	—	—

[1] Varieties: Oderbrucker, Wisconsin Barbless, Velvet, Manchuria, and Trebi.
[2] Stations: East Lansing, Mich., Madison, Wis., Waseca, Minn., Kanawha and Emmetsburg, Iowa, Brookings, S. D., and Bozeman, Mont.

1935, and 1938 than in the dry season of 1936, and in 1937 when stem rust was severe at four of the stations.

The average protein content of the barleys and malts showed varietal, seasonal, and station differences. Trebi ranked lowest in protein content, while Wisconsin Barbless, Velvet, Manchuria, and Oderbrucker following in ascending order. The last three varieties were relatively similar and occasionally changed rank in the yearly and station averages. The average protein content for 1935–38 at the six stations was relatively similar, while the 1934 average was appreciably higher.

The average hull contents of the five varieties were similar except for Manchuria which was lower than the other varieties. The average for the five years varied from 10.9 in 1935 to 15.8% in 1937, the season that stem rust was severe on barley through the spring barley area. The average hull content for 1938 was somewhat higher than for the first three years.

The average recovery of malt from barley showed a varietal and seasonal difference. The varietal ranking for recovery arranged in descending order was as follows: Trebi, Wisconsin Barbless, Velvet, Manchuria, and Oderbrucker; the latter two varieties were not significantly different in recovery. Average recovery was lowest in 1935, and highest in 1936 and 1937.

Average bushel weight and kernel weight of malt showed a fair agreement with bushel weight and kernel weight of barley in both the varietal and seasonal averages. The seasonal averages for bushel weight of malt showed greater variations than in the averages for the varieties. The average kernel weight of malts from the six stations indicates three varietal groups: Trebi 30.6 mg., Wisconsin Barbless 26.8 mg., and Oderbrucker, Velvet, Manchuria 23.1, 22.6, 22.0 mg. respectively. The average kernel weights of malt in 1934 and in 1938 were significantly higher than in the other three years.

The average extract content showed varietal, station, and seasonal differences at the six stations. The varietal ranking in ascending order was as follows: Wisconsin Barbless, Velvet, Oderbrucker, Trebi, and Manchuria. The varietal ranking of the last three varieties varied slightly over the larger number of stations and in individual years, but this variation was not significant. The average extract content for the five varieties combined for each of the five years indicated that the seasons of higher moisture, namely 1935 and 1938, were the more favorable for the production of high extract.

The average soluble nitrogen in the worts and in percentage of the total malt nitrogen showed marked varietal, station, and yearly differences. Both Wisconsin Barbless and Trebi malts were significantly lower than the malts of the other three varieties. The lower values for these two varieties have been consistent at the different stations and in all years. The other three varieties changed positions at the different stations, and in different years also showed differences, in that 1934 was low and other years were intermediate or high in soluble nitrogen as percentage of malt nitrogen.

The averages for diastatic power of the malts suggested the same general varietal, station, and seasonal responses as shown for soluble nitrogen in the wort as percentage of malt nitrogen and as shown for conversion time. The same two varieties, Wisconsin Barbless and

Trebi, were significantly lower than the other three. The average diastatic power for the season of 1938 was appreciably higher than for the previous three years (1935–37) in which the seasonal averages were very similar. The low average for 1934 was caused mostly by the method of drying the malts in which the moisture content was reduced to an average of 2.9%.

In summation, the malts from the barleys grown in 1938 at all stations were in general of uniformly better quality than in any of the previous years included in the regional study. The 1938 season perhaps represents the first year in the five when growing conditions were favorable over the entire area represented by the cooperating stations. There have been two years at least at each station when seasonal conditions have been favorable for the development of good-quality barley. There likewise have been two years at most of the stations in the area when the growing conditions have been very unfavorable for barley production. The five-year survey has shown that regardless of these wide variations in environmental conditions the varieties maintain their relative ranking, except as noted, for most of the characteristics used in evaluating quality.

BOOK REVIEWS

A Physiological Study of the Winter Wheat Plant at Different Stages of Its Development. By Edwin C. Miller. Technical Bulletin No. 47 of the Kansas Agricultural Experiment Station, Manhattan, Kansas. 167 pages. 1939.

This extensive bulletin deals with studies of certain phases of the metabolism of the nitrogen, phosphorus, potassium, and carbohydrates of the winter wheat plant from "seed to seed." Whereas much of the published data on this subject is representative of limited analyses performed during one growing season, Dr. Miller presents in this bulletin the results of adequate sampling throughout four crop years. The appendix of 84 pages of tables gives some idea of the scope of this investigation. For additional clarity full use of figures is made throughout the text. Climatological data and an adequate bibliography of 92 literature citations make the bulletin a complete presentation.

Analyses were made on the grain before planting early in October, then on the growing plant at two- or three-week intervals throughout the winter. After initiation of rapid "spring growth" in April sampling was conducted at weekly intervals until the plants were mature with ripe grain in June. During the last growth period of grain development and ripening, data are presented for the chaff and grain as well as for the stems and leaves. The data include analyses for total and protein nitrogen, total and water soluble phosphorus, total potassium, reducing and nonreducing sugars, starches, and hemicelluloses. All analyses are expressed both on the basis of percentage of dry weight and as grams per 100 plants. Some might wish that certain constituents, for instance the water-soluble carbohydrates, were expressed on the basis of green weight or as percentage in the water present in the growing plant. However the adequate data permit recalculation by those particularly interested in this phase.

Dr. Miller's very comprehensive work will be of great value not only to the physiologist interested in the metabolism of the winter wheat plant throughout the early periods of its development but likewise to the cereal chemist whose interest is perhaps more strongly directed to the elaboration of proteins and carbohydrates in the ripening grain.

ERIC KNEEN

Physical Tests of Dough Quality. By C. H. Bailey. Published as Volume XVI, No. 6, of Wheat Studies, by the Food Research Institute, Stanford University, California. March, 1940. 57 pages with illustrations. Price $1.25.

This is a thoroughgoing account of what has been accomplished to date in the application of mechanical techniques for the characterization and measurement of those physical dough properties that are of major interest and importance to the cereal technologist. There are 129 literature citations.

Divided into three sections, the first deals with flour absorption in relation to properties such as viscosity, mobility, elasticity, tensile strength, extensibility and stickiness. These properties, respectively, are defined and given mathematical expression wherever the latter is possible. Most of the methods and devices that have been proposed at various times for the estimation of water absorption are described, including viscometers, plastometers, extensimeters, dough mixers, etc.

The second section discusses physical tests of crude gluten, describing specifically the several types of apparatus and procedure (starting with Boland in 1848) that have been devised and recommended for the characterization of gluten properties. The reader is cautioned that gluten, after all, is only one dough constituent, and that its properties may not always necessarily serve as a completely satisfactory index to dough characteristics.

The largest of the three sections is the one on physical tests of wheat-flour dough. This is divided into subsections dealing, respectively, with recording dough mixers, ductility measurements, extensimeters, and the extensograph. The principles underlying each method and instrument are fully explained, and the more important of these are illustrated by pictures, diagrams, and charts. The author considers it highly unlikely that any one instrument or any single test can ever be expected to serve as a basis for the complete appraisal of dough quality and baking behavior. "Rather must we expect, for some time to come, that several tests must be applied, notably through the progress of a fermentation period, to determine how a dough is responding with the lapse of time, and in the face of ensuing biochemical events."

In the preparation and publication of this monograph, both the author and the Food Research Institute have rendered a noteworthy and timely service to cereal technologists.

M. J. BLISH

World Wheat Planning, and Economic Planning in General. By Paul de Hevesy. Oxford University Press, London. 910 pages. Price 38/–.

Of this large volume, only the prospectus of which has been made available to the reviewer, Part I states the world wheat problem with its implications, discusses the economic factors affecting it, and proposes a world plan involving price agreement, acreage adjustment, disposal of surpluses, etc. Part II discusses the merits of the competitive system versus state planning and control of goods and services in general and in the light of specific economic conditions and trends. The future of agriculture is dealt with. Part III takes up the agricultural policies of 47 individual nations, respectively.

The book is represented to be a unique and comprehensive encyclopedia of factual data on wheat production, acreage, yield, trade consumption, stocks, transport, costs, prices, and sources of information. These data are presented in 56 appendices covering some 200 pages.

M. J. BLISH